REPORT OF THE THIRTY-THIRD NORTH AMERICAN ALFALFA IMPROVEMENT CONFERENCE

June 14-18, 1992
Holiday Inn/Airport - North
Atlanta, Georgia

NAAIC

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The Thirty - Fourth North American Alfalfa Improvement Conference will be held on:

July 10 - 14, 1994
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THIRTY - THIRD NORTH AMERICAN ALFALFA IMPROVEMNT CONFERENCE

Program Chairman - Gary Bauchan

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INTRODUCTION

The 33rd North American Alfalfa Improvement Conference held at Atlanta, GA, included two and one-half days of paper and poster sessions and business meetings, a half-day scientific tour of the University of Georgia Central Georgia Branch Station, and a full-day post-conference tour of the University of Georgia Coastal Plain Experiment Station at Tifton, GA, the USDA facilities at Byron, GA, and the Masstock Dairy Farm. An evening industry supported awards banquet recognized Donald C. Erwin, Bernard P. Goplen, Darrell A. Miller, Donald K. Barnes, and John E. Baylor as 1992 NAAIC Honorary Members and Barbara W. Pennypacker as the R. R. Hill Award winner. Bobby Rowan, Georgia farmer and Chairman of the Public Works Commission, State of Georgia gave a presentation on some of the local life in Georgia during the banquet.

This report includes abstracts of paper and poster sessions, committee reports, history and information on distribution of conference reports and membership. Contributing authors and their organization are responsible for the information they present in this report. Those wishing to reproduce any part of this report should consult the author.

Participation in the conference by workers in all disciplines of alfalfa science was excellent. The 27th Forage Insect Workers Conference and the NCR 138 Plant Pathology Committee also met during the conference.

Copies of this report and those of the 1982, 1984, 1986, 1988, and 1990 conferences are available at $10 each from the NAAIC Executive Secretary, James H. Elgin, Jr., USDA/ARS/NPS, Bldg. 005, Rm. 328, 10300 Baltimore Avenue, Beltsville, MD 20705-2350. Checks should be made payable to NAAIC.

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Overview of a Decade of Medicago Interspecific Hybridization Research

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Research has focused on producing interspecific hybrids between alfalfa and other perennial *Medicago* species, and subsequent use of the hybrids in alfalfa breeding and genetics. It is now possible to recover hybrids between alfalfa and all other species of the subgenus Medicago. Successful hybridizations fall into two categories: 1) hybrids recovered from seed and 2) hybrids that require ovule-embryo culture (1).

The first group includes crosses with *M. sativa*(2x) and *M. glomerata*(2x) and *M. prostrata* (2x), as well as, *M. sativa*(4x) crossed with the following species: *M. cancellata*(6x), *M. saxatilis*(6x) and *M. glutinosa*(4x). Group one also includes the unique combinations of *M. sativa*(2x) x *M. dzhawakhetica*(4x) and *M. sativa*(2x) x *M. papillosa*(4x). Uneven ploidy levels are essential for recovering hybrids from these crosses, and all progeny are triploid. Ovule-embryo culture is required to produce hybrids between *M. sativa*(2x) and the following species (in order of increasing difficulty in hybrid recovery): *M. rhodopea*(2x), *M. rupestris*(2x), *M. dzhawakhetica*(2x), *M. papillosa*(2x), *M. daghestanica*(2x), *M. pironae*(2x), *M. hybrida*(2x), *M. suffruticosa*(2x) and *M. marina*(2x). In addition 4x hybrids can be recovered from *M. sativa*(4x) crossed with 4x accessions of *M. dzhawakhetica* and *M. papillosa.*

Of the various hybrid combinations studied to date we have found *M. dzhawakhetica* and *M. papillosa* offer the most potential. *M. dzhawakhetica* has a crown and root morphology that appear to confer excellent winterhardiness. Selecting for this trait in backcross (BC) generations with *M. sativa* results in plants capable of withstanding severe winterkill. *M. dzhawakhetica* has also been reported to be resistant to spring blackstem and Verticilium wilt. Furthermore yield studies on 15 BC2 families showed that four of these families outyielded the varieties used in backcrossing. *Medicago papillosa* also has excellent potential for alfalfa improvement. One method we have been exploring is to produce alloautohexaploids with this species. These novel hexaploids are chromosomally stable (2) and may be useful in some environments. Although these two species offer novel germplasm there are significant hurdles to circumvent including: the requirement of using uneven ploidy levels, limited genomic affinity between alfalfa and the wild species genomes (3), limited seed set with the first backcross (because the F1 is triploid) and most of the first backcross progeny are pentaploid.

Medicago rhodopea(2x) and *M. rupestris*(2x) hybrids with 2x alfalfa have been extensively studied. Tetraploid hybrids produced by somatic doubling with colchicine have also been examined. Yield studies have been conducted on *M. sativa x M. rhodopea* backcrosses with *M. sativa.* At the 2x level three of 20 BC1 families outyielded the check. At the 4x level, 6 of 12 BC1 and 3 of 11 BC2 families outyielded the check. Yield analysis was not conducted on the *M. sativa x M. rupestris* backcross generations because we observed significant chromosome instability in the first backcross, where more than 25% of the BC1 progeny were aneuploid. Although the potential of *M. rhodopea* and *M. rupestris* is not as great as for *M. dzhawakhetica* and *M. papillosa,* all four of these species can be considered potential germplasm donors for alfalfa improvement. The other hybrid combinations mentioned in group 2 will be much more difficult to utilize. At this point only sterile hybrids have been recovered between *M. sativa*(2x) and the following: *M. daghestanica, M. pironae, M. hybrida, M. suffruticosa* and *M. marina.*

References

1. McCoy, T. J. and Smith, L. Y. 1986. Theor. Appl. Genet. 71:772-783.
2. McCoy, T. J. 1989. Genome 32:302-306.
3. McCoy, T. J., Echt, C. S. and Mancino, L. C. 1991. Genome 34:574-578.

Alfalfa Germplasm and the Alfalfa Crop Advisory Committee

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The Alfalfa Crop Advisory Committee (ACAC) provides advice and leadership on issues pertaining to *Medicago* germplasm in the U.S. including germplasm collection, seed increase, and evaluation. Members of the ACAC are appointed by the President of the NAAIC and represent specific regions within the U.S., scientific disciplines, or industry groups. Most of the committee's activities ultimately focus on administration of the U.S. Plant Introduction (PI) collection of perennial and annual *Medicago* species. This collection, held at Pullman, WA, contains nearly 2300 accessions of *M. sativa*, 387 accessions from other perennial *Medicago* species, and 2260 accessions of annual *Medicagos*. Activities of the committee are concentrated in four basic areas: 1) Prioritizing exploration and acquisition; 2) Management, description, and evaluation of the collection; 3) Recommending on personnel, facilities, and research needs; and 4) Promotion and assessment of germplasm use. Recent ACAC activities related to collection management, and evaluation are described below.

Since 1983, the ACAC has overseen a program to evaluate introductions of *M. sativa* for traits that could be of value to germplasm users. A wide variety of traits have been evaluated, including responses to many insects, diseases and abiotic stresses. Data from evaluations is most readily accessible to users through the GRIN (Germplasm Resources Information Network) computer database. A total of over 54,000 entries (accessions X evaluation values) have been made into GRIN for *M. sativa*.

Evaluation data has been used by researchers to improve the usefulness of the *Medicago* PI collections. For example, Don Barnes and coworkers (USDA-ARS, St. Paul, MN) have used evaluation data to designate a reduced set of accessions of *M. sativa* that are representative of the range of diversity within the entire collection. This "core collection", which contains 200 accessions, should provide more efficient access to the collection, as well as streamline the maintenance of the entire collection. Gary Bauchan (USDA-ARS, Beltsville, MD) has also led the development of a core collection containing 204 accessions of annual *Medicagos* from 35 species.

In order to better serve the users of germplasm the ACAC sent a Germplasm Use Questionnaire to NAAIC members in North America in early 1992. A total of 102 questionnaires (28%) were returned. One component of the questionnaire dealt with the general types of traits that potential germplasm users might hope to locate in plant introductions. Over half of respondents reported they might use *Medicago* germplasm from the PI collection as sources of resistance to insects (55% of respondents) or diseases (52%). The next most commonly cited uses were as sources of increased persistence (40%), and forage quality (38%), and novel physiological traits (36%). Of the respondents, 53% had actually utilized *Medicago* PIs in their work. Nearly half of these respondents (47%) reported they identified the accessions utilized from printed catalogs. Only 25% had located the germplasm they utilized using GRIN, the most complete and up-to-date source of information on this germplasm.

Respondents were also asked to identify specific traits that should be evaluated in the PI collection. Nearly all responses concentrated on traits of importance in perennial *Medicagos*. Most commonly listed was resistance to potato leafhopper (34% of respondents), alfalfa weevil (24%), clover root curculio (24%), and stem nematode (15%), and improved forage quality (15%), salinity resistance (15%), and acid soil resistance (13%). Of these seven traits, evaluation data for *M. sativa* already exists on GRIN for all but two (Table 1). This highlights the need to improve access to evaluation data, especially through GRIN. Persons interested in receiving a GRIN usercode and instruction manual should contact John Belt, USDA/ARS, Room 118, Bldg. 001, BARC-West, Beltsville, MD 20705, (301) 504-5145. Those interested in receiving seed or information about the *Medicago* collection should contact Dave Stout, USDA/ARS, 59 Johnson Hall, Washington State Univ., Pullman, WA 99164, (509) 335-1502.

Table 1. Number of *M. sativa* accessions evaluated for traits questionnaire respondents most commonly cited as deserving evaluation.

Trait	Accessions in GRIN
potato leafhopper resis.	739
alfalfa weevil resis.	0
clover root curculio resis.	556
stem nematode resis.	0
forage quality	1097
salinity resis. (germ.)	1301
(mat. plt.)	881
acid soil resis.	352

Exploring for Medicago ruthenica in Inner Mongolia

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The Alfalfa Crop Advisory Committee of the National Plant Germplasm System determined that there was a need for *Medicago ruthenica* in the System for improving the stress tolerance of cultivated alfalfa. An exploration for this species was conducted in Inner Mongolia from August 15 to September 19, 1991. During the expedition, seed and root-environment soil samples from 101 *M. ruthenica* populations were collected near the cities of Hohhot, Tongliao, and Xilinhot. These are ecologically diverse sites within temperate steppe and temperate desert steppe regions. Four populations identified as *Medicago platycarpa* were found bordering *Populus* sp. plantings at elevations approaching 1300 m. Seed accessions are currently undergoing increase in the US, and soil accessions are awaiting export clearance at the Soil and Fertilizer Institute in Beijing. Among the associated genera found in the temperate steppe regions were *Agropyron, Agrostis, Allium, Ambrosia, Artemisia, Astragalus, Caragana, Hedysarum, Leymus, Medicago sativa* sp. *sativa* and *falcata, Polygonum, Populus, Potentilla, Setaria,* and *Stipa.* Some associated genera observed in the temperate desert steppe areas were *Agrostis, Artemisia, Aster, Digiteria, Lespedeza, Leymus, Quercus, Setaria, Stipa, Xanthium,* and some unidentified small trees. We collected seeds from one *Avena*, one *Agropyron,* two *Astragalus,* three *Hedysarum,* seven *Medicago sativa* spp. *falcata,* nine *Medicago sativa* spp. *sativa,* and one *Triticum* populations.

Most of the *M. ruthenica* accessions were collected under heavy grazing pressure and/or heat and drought stress. These genetic resources appear to be in danger of loss due to mismanagement of their native environment. Most *M. ruthenica* plants exhibited some grazing tolerance due to a procumbent growth habit and tillers that could reach 1 m in length. Erect plants were also noted and appeared to be associated with reduced grazing pressure, although procumbent and erect plants could be found growing in the same location. We found very few nodulated plants. *Medicago ruthenica* appeared to be quite drought tolerant, and we found little evidence of insect damage on either the foliage or roots, although some appeared to have nematode damage. Various insects parasitized the seed pods. Many of the *M. ruthenica* plants sampled were at least four years old and exhibited crown rots, but no variation in response to this disease was noted. Although most pods were dehiscent, there appeared to be variation for both time of suture opening and the ability to retain the seed in the open pod. We concluded that the performance of much of the material collected during the exploration would improve markedly under adequate moisture and fertility. Most local herdsmen were aware of the species and indicated that it was a desirable plant for livestock production.

Medicago ruthenica is often much less prevalent at the top or bottom of a slope than in the middle. This distribution pattern seems to be fairly ubiquitous in the areas we explored. We concluded that *M. ruthenica* is limited on the upper slopes by exposure and drought, while on lower slopes it is limited by excess moisture and/or competition from other species. Additionally, seeds produced by the upper slope plants, where vegetation is spare, may be washed downhill by heavy runoff.

We were alarmed to learn that there may be no areas in Inner Mongolia (perhaps in the entire PRC) which are comparable to US reserves or national parks. Essentially all of the grasslands are mowed and/or heavily grazed before most of the pods ripen. This provides little opportunity for *M. ruthenica* (and many other species) to reproduce successfully. We believe that germplasm collections in these "at risk" areas should be completed as rapidly as possible.

Development of an Annual Medic Core Collection

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The annual medics are endemic to the Mediterranean region of the world. The annual medics are fast growing, produce a large amount of biomass with a large number of pods. 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. The annual medics are used extensively in Australia were they are utilized to improve soil structure, increase soil nitrogen and as a source of winter forage. The species most widely grown in Australia are: *M. littoralis*, *M. polymorpha*, *M. rugosa*, *M. scutellata*, *M. italica*, and *M. truncatula* (Crawford, 1989).

There are 34 recognized species of annual medics and 3159 accessions of the annual medics in the U. S. Plant Introduction collection. Although there is interest in the annual medics for use in sustainable agricultural systems, the U. S. collection is under utilized due a lack of agronomic information. Development of a core collection may be a method of condensing the collection of agronomic data on the available germplasm to make it more accessible for utilization by breeders and agronomists. A core germplasm collection is a small collection that represents the maximum genetic diversity with minimum repetitiveness.

During the summer of 1990 we grew 1220 accessions of the annual medics in the field at Beltsville, MD for selection of a core collection. The criteria for choosing accessions within a species for evaluation were first the place of origin, i. e. accessions were chosen to represent proportionally the countries of origin in the total collection, and secondly the availability of seed. The accessions were evaluated for: 1) days to flower, 2) days to full pod, 3) biomass within a species, 4) biomass between species, 5) growth habit, 6) pod production, 7) pod spines, 8) plant height, 9) plant width, 10) length of middle leaflet, 11) width of middle leaflet, 12) internode length, 13) number of flowers per raceme, and 14) number of pods per raceme. Accessions were selected for the core collection using cluster analysis (an unweighted pair group method using arithmetic averages). The resultant 212 accessions, contains all 34 species and has one accession per cluster within a species.

The species which appear to have the greatest potential use in the U. S. based on our evaluations at Beltsville, MD are: M. scutellata, M. blancheana, M. italica, M. polymorpha, M. rugosa, and M. lupulina. These species produced the largest amount of forage during the spring and summer months (June through September). The species which flowered the earliest were M. scutellata [(47 days after planting (dap)], M. blancheana (50 dap), M. laciniata (57 dap) and M. rotata (57 dap). Several species did not flower before a killing frost occurred.

The significance of developing the medic core collection are: 1) the core contains a majority of the genetic variability which exists in the entire collection, 2) the process of developing a core identified species which need additional germplasm collections, 3) the core will make future evaluation of the annual medics more efficient, and 4) the core has increased the utilization of the medic germplasm collection.

Further evaluations of the core are being conducted in seven locations (Athens, GA; Beltsville, MD; Ithaca, NY; Logan, UT; Pullman, WA; and St. Paul, MN; Tucson AZ) to determine if there are genotype X environmental interactions and to allow researchers and interested individuals an opportunity to observe the annual medics. Evaluation data will be entered into GRIN. There is a limited amount of seed available of each accession in the core collection. Seed can be obtained by contacting Dr. Richard Johnson, USDA/ARS, P. I. Station, Washington State University, Pullman, WA 99164.

References

Crawford, E. J., A. W. H. Lake, and K. G. Boyce. 1989. Breeding annual *Medicago* species for semiarid conditions in southern Australia. Advance in Agronomy. 42:399-437.

Inheritance of Tap, Secondary, and Fibrous Root Traits in Alfalfa

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Knowledge about the inheritance of root traits could help determine selection procedures for developing alfalfa populations with specifically adapted root types. McIntosh and Miller (1981) studied root branching using a diallel cross. General combining ability (GCA) was important at one location, while specific combining ability (SCA) was important at another location. Pederson (1982) studied several root traits using a six-cultivar variety cross diallel. GCA was significant for root dry weight, root diameter at the crown, root diameter 15 cm below the crown, number of lateral roots, and taproot branching. SCA was significant only for root diameter 15 cm below the crown. The objectives of this research were to determine the inheritance of alfalfa for seven root morphological traits, to determine the effect of plant spacing and location on these traits, and to correlate root traits with dormancy and forage yield. Progenies of three, six plant diallels, and six, 4 x 4 and seven, 3 x 3 design II mating designs were evaluated. They were planted at Rosemount and at Becker, MN, in 30 cm rows with 2.5 cm between plants within the row and 1 m between rows. The plots were evaluated for yield and fall dormancy. In late October, the plants were dug and evaluated for taproot diameter (TD), secondary root number (SN), secondary root diameter (SD), and secondary root position (SP), fibrous root mass (FIB), percent determinate taproots (PD), and determinate taproot position (DP). Because plants from the ends of rows had more space to grow they were evaluated separately from plants in the middle of the rows. Plant spacing effected root morphology, but few genotype X position within plot effects were observed. Location affected root morphology,, but few genotype X location effects were observed. General combining ability (GCA), but not specific combining ability (SCA), was significant for TD, SN, SD, SP, FIB. These traits had moderate to high heritabilities of 0.53, 0.67, 0.64, 0.64, and 0.81, respectively, based on half-sib family means from design II analyses. Neither GCA nor SCA was significant for PD and DP. These traits had low heritabilities. TD and SD were the root traits most correlated with forage yield. Selecting for increased TD, SN, SD, SP, or FIB at either location using uniform (either spaced or solid seeded) plots should be effective. More research is necessary to find an environment in which selection for PD and DP could be effective.

References

McIntosh, M. S., and D. A. Miller. 1981. Genetic and soil moisture effects on the branching-root trait in alfalfa. Crop Sci. 21:15-18.

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Taproot Protein Reserves and Performance of Alfalfa

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Total nonstructural carbohydrates (TNC) in taproots of alfalfa are thought to be essential for growth in spring and regrowth after harvest, but we have observed little association between genetic differences in taproot TNC concentrations and alfalfa regrowth (1, 2, 3, 4). We recently initiated studies focusing on other taproot constituents that may be important in alfalfa growth and stress tolerance, including taproot proteins. Results from a study where plots were sampled at ca. three-week intervals from fall to late spring indicate that taproot protein concentrations increase in late fall as plants harden for winter [(Fig. 1), 5]. Like TNC, taproot protein concentrations decline extensively in spring as plants resume growth. Defoliation in summer also results in declines in both taproot proteins and soluble amino acids (Fig. 2). In both studies use of taproot proteins occurs during periods when dinitrogen fixation is very low (early spring, immediately after defoliation). At these times mobilization of taproot proteins and amino acids occurs to meet the N needs of growing shoots until dinitrogen fixation resumes after which taproot protein levels return to normal concentrations (Fig. 2, Days 25+).

Several specific taproot proteins are very abundant in taproots of alfalfa and these are preferentially used as sources of N during growth in spring and regrowth after harvest. These vegetative storage proteins (VSP's) are found only in alfalfa taproots (not in seeds, stems, leaves, or nodules) and appear to be unique to alfalfa (they are not found in taproots of trefoil, clovers, and sweetclover). We have used ineffective nodulating alfalfa mutants that do not accumulate VSP's to verify their importance in shoot regrowth. Fertilizing ineffective plants with N during late regrowth stimulates VSP accumulation. When transplanted into a low-N environment, ineffective plants with VSP's have twice the shoot regrowth rate when compared to ineffective plants lacking VSP's. Future work will focus on regulation of expression of VSP genes, localization of VSP's within cells, and the distribution of VSP's among annual and perennial *Medicago* species.

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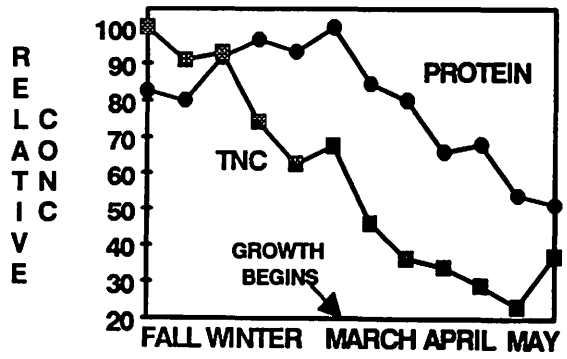

Figure 1. Trends in total nonstructural carbohydrate (TNC) and protein in alfalfa taproots between Sept. and May.

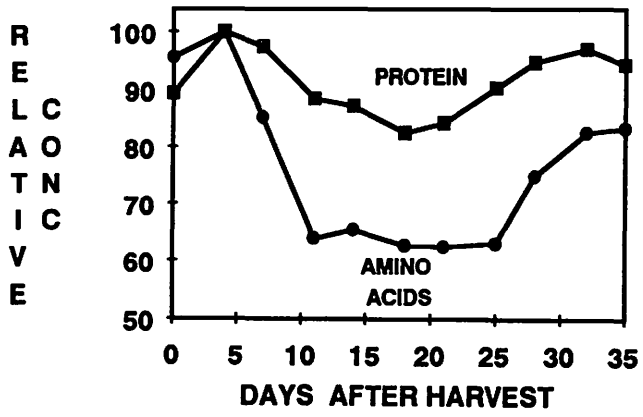

Figure 2. Protein and amino acid concentrations of alfalfa taproots during regrowth after defoliation.

Inheritance of Crown Morphological Characteristics in Alfalfa

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Information about the inheritance of crown morphology in alfalfa can be valuable for deciding which selection methods to utilize when developing populations with specific crown morphology traits (Marquez-Ortiz et. al., 1991). For example, the crown morphological characteristics: crown area, number of stems and number of crown buds, were associated with persistence and grazing tolerance in alfalfa (Brummer and Bouton, 1991). Most studies about the alfalfa crown have been limited to crown diseases and little information exists about the inheritance of crown morphological traits. The objectives of this study were to determine the inheritance of crown morphological characteristics in alfalfa, to determine the effect of plant spacing and location on crown morphology, and to determine the relationship between crown morphology and forage yield.

Progenies of six 4x4 and seven 3x3 design II mating designs were grown at Rosemount and Becker, MN from May to October of 1991. All plants were direct seeded in 30 cm-row plots with 2.5 cm between plants within the row and 1 m between rows. Forage yield was measured in three harvests during the growing season. All plots were scored for fall dormancy in October 1991. In late October, the plots were undercut to a depth of 25 cm. The plants were pulled, washed and photographed. Crown depth (CD), crown width (CW), number of crown stems (NCS), crown stem width (CSW), and number of crown buds (NCB) were evaluated from the photographs using 1(=low) to 5(=high) scoring systems. Plants at both ends of each plot were evaluated separately from the center 10 plants to account for plant space effects on the traits measured.

Plant spacing affected crown morphology. Small entry x spacing interactions were observed. Location affected crown morphology: crowns were deeper at Becker (sandy soil) as compared to Rosemount. All the other traits had higher means at Rosemount compared to Becker. Entry by location effects were only significant for NCB ($P > 0.01$). Additive and dominance variances were both significant for CW and NCS. Additive variance was higher in magnitude than dominance variance. Only additive variance was significant for CD, CSW, and NCB. CW, NCS, and CSW had moderate heritability values of 0.36, 0.50 and 0.45 respectively, based on half-sib family means from the design II analysis. CSW was the only crown trait correlated with fall dormancy ($r=0.68$). Correlations between crown characteristics and forage yield were low. Selection for increased CW, NCS, and CSW should be effective at both locations utilizing either spaced or solid seeded plots.

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Differential Cold-tolerance Between Alfalfa Crown and Root Tissues

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Winterhardiness is a major factor limiting the persistence of alfalfa stands in northern climates. The complex nature of the trait makes it difficult to evaluate and to predict. Cold-tolerance, fall dormancy, pest resistance and harvest management may all influence the winterhardiness of a cultivar. It is difficult for a single indirect test to consider all of these factors. Cold-tolerance is one of the major factors contributing to winterhardiness. Schwab et. al. (1991) developed a laboratory test designed to test the cold-tolerance of mature alfalfa plants by measuring recovery following freezing. The objective of the present research was to further standardize the test methodology and to compare the cold-tolerance of crown and root tissues for alfalfa cultivars.

Ten alfalfa cultivars were seeded at Rosemount, MN in 5.5 m rows 1 m apart in early June of 1991. The cultivars were '5262', '5432', 'Arrow', 'Commandor', 'DuPuits', 'Epic', 'Magnum III', 'Moapa 69', 'Profit', and 'Spredor 2'. Plants were mowed in July and September. They were dug after undercutting to a 25 cm depth in early November. Plants were washed, clipped at 5 cm above the crown and 10 cm below the crown, bundled into groups of five, and stored at -2° C. After digging, the plants never left the -2° C cold room. One bundle of each entry was packaged into a 12x6x6 cardboard box containing 10 cm of dry vermiculite. The boxes were placed in a controlled freezing chamber where the temperature was lowered 4° C every 1.5 hours. Boxes were removed from the freezer following -12, -16, -20, and -24° C treatments. Following freezing, the plants were thawed for 12 hours at 4° C. Plants were planted in greenhouse sandbenches and allowed to regrow for 3 weeks. Roots and crowns were scored separately for injury and recovery.

Alfalfa cultivars differed in root and crown survival scores at -12° C, and -16° C, but at -20° C there were no significant differences among cultivars for either root or crown survival scores. At -24° C all plants were dead. Although average root survival scores were correlated with average crown survival scores ($r=0.93$ $P<0.001$), cultivar rank for crown survival was not the same as cultivar rank for root survival. Crown and root survival scores decreased linearly with decreasing temperatures for most cultivars; however, 5262, Magnum III and Commandor crown survival scores had a quadratic response to decreasing temperatures. Change of crown survival scores with decreasing temperatures differed among cultivars and was related to average crown survival score ($r=0.92$ $P<0.001$). Change in root survival score with temperature was not as strongly associated with average root survival score ($r=0.67$ $P<0.05$). This evidence suggests that alfalfa cultivars have differential responses to a freezing stress, and that root and crown tissue respond differently.

Average cultivar crown survival scores from the laboratory experiments were correlated with the survival (scored in May 1992) of overwintered plants in the field at Rosemount, MN ($r=0.68$ $P<0.05$). Field survival was not significantly correlated with the average root survival score from the laboratory experiments. The ability to regrow from below a damaged crown seemed to be an important trait for survival in both the laboratory and field studies. The correlation of the laboratory test with field survival suggests that this should be a useful method for laboratory testing of alfalfa cold-tolerance.

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Variation between alfalfa cultivars for mineral content

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Routine tissue analysis of alfalfa from across Manitoba shows that most is marginal or deficient in copper for cattle feed and marginal or low in sulphur for alfalfa growth. A study was therefore initiated to determine if plants differ in their level of these elements, or in their ability to grow well on a low S supply.

Plant samples were taken from an existing alfalfa test, growing on a clay-loam soil and fertilized with annually with P and S. The soil had adequate levels of most of the minerals, although the S was marginal. Individual plants were sampled on 9 June, 5 days before the first harvest, by cutting 5 cm above ground level. Four plants of each of 35 cultivars were analyzed for N, P, K, S, Ca, Mg, Zn, Fe, Mn and Cu.

Despite the small sample size, cultivars differed significantly for P, K, S, Ca, Mg and Cu (Table 1). Those cultivars with the greatest proportion of falcata background, including Ufimovskaya 7, Drylander, Rangelander and Rambler, had the lowest concentrations of S. A comparison of alfalfa populations had similar results (2). High S cultivars included Husky, Blazer and Nitro. All plants were within the range of S required for normal growth. Unusually, the falcata types tended to have the highest yield, which was negatively correlated with S content.

The concentration of Cu was highest in the cultivars Primal, AP 40, Victoria and Nitro, each having over 6 $\mu g.g^{-1}$. Angus, Rangelander and WL 222, by contrast, had about half that concentration. The level of Cu in forage needed to meet the animal's requirements is 5-10 $\mu g.g^{-1}$ (1) which was not met in nearly half of the cultivars, and the remainder had marginal levels.

The variation between cultivars in Cu content, taken with the lack of correlation between Cu content and yield, suggests that farmers in areas prone to Cu deficiency in forage could alleviate this by selecting an appropriate cultivar. Currently, further samples are being analyzed to confirm these findings and to provide information for recommendations.

Table 1. Variation in mineral content of selected alfalfa cultivars.

	yield $t.ha^{-1}$	P	K	S	Ca	Mg	Cu $\mu g.g^{-1}$
				$mg.g^{-1}$			
	***	**	***	*	**	**	***
Husky	3.7	2.54	30.5	3.40	17.3	3.46	5.53
Blazer	4.2	2.47	30.1	3.32	13.7	3.09	5.61
Nitro	1.8	2.63	30.1	3.32	14.8	3.26	6.06
Rambler	4.4	2.34	30.8	2.56	15.1	3.48	4.84
Drylander	5.1	2.45	32.6	2.55	14.8	3.08	5.25
Ufimovskaya 7	4.9	2.28	27.9	2.50	12.9	3.16	4.89
Primal	3.6	2.39	28.4	3.18	16.3	3.45	6.43
AP 40	4.3	2.75	34.1	3.08	15.6	3.46	6.32
Victoria	4.4	2.53	27.7	3.15	13.9	3.09	6.11
WL 222	3.5	2.32	32.5	2.84	14.9	3.26	3.56
Rangelander	4.8	2.49	33.1	2.56	15.4	2.74	3.11
Angus	3.5	2.61	32.8	2.93	14.1	2.91	3.04
SE	0.2	0.12	1.1	0.20	0.89	0.21	0.40

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Utilizing 28 Years of Alfalfa Variety Trials Data For Extension Programming. "Cards to Computers"

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Alfalfa variety trials have been conducted at the University of Illinois for many years and have been reported annually in the Forage Crop Variety Trials in Illinois report. The data reported include (a) individual harvest dry matter yields, (b) % of Check Variety yield for annual yield and multiple year averages, and occasionally (c) vigor, stands, insect, and disease ratings.

An Index Card (10.16 x 15.24 cm) record system was initiated in 1963. Each variety in trial had a card and each card had data of all trial sites and years for the variety. The data recorded was % of Check Variety Yield. Check varieties were: Atlantic, Buffalo, Ranger and Vernal.

Check varieties selected in late 1960's were: Atlantic, Buffalo, Cody, Narragansett, Ranger and Vernal.

In the early 1970's, the Central Alfalfa Improvement Conference suggested uniform "Check Varieties" for alfalfa variety trials in the Central Region of the United States. The varieties suggested were: Dawson, Kanza, Saranac and Vernal.

In 1979, the "Check Varieties" selected were: Baker, Riley, Saranac AR and Vernal and have been used for "checks" through 1992.

A computer with Lotus 123 spreadsheet replaced the Index Cards in 1984. The filing system identified 3 geographic and climatic regions of Illinois: Northern, Central, Southern. The regional system (a) reduced the size of files, (b) speeded retrieval of data, and (3) facilitated data use on a regional basis for more site specific variety selection suggestions for extension clientele.

Each variety in a Regional Trial has an entry line in the spreadsheet. Each data entry of % of Check Variety Yield is entered on a separate line with other data entries of that variety for Production Year Yield or Seeding Year Yield. All data of a variety are grouped, line by line on the spreadsheet and the % Check Average is calculated. Seeding year yields are not used to obtain the % Check Average.

The Extension use of the data has been to rank varieties by yield, searching for top performing varieties. The criteria for ranking includes: (a) 3 years or more of production after seeding year in the Region to assess winter survival and persistence of yield; and (b) a statement from merchants that the variety will be marketed in Illinois during the ensuing marketing year.

Energy Exchange of Dormant and Nondormant Alfalfa Types

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Dormant alfalfa types have lower canopy temperatures (Tc) and higher leaf stomatal conductance than nondormant alfalfa types (Hattendorf et al. 1990). Higher transpiration per unit leaf area indicates intrinsic differences between the dormant and nondormant alfalfa types, which may lead to differences in evapotranspiration on a canopy basis. The purpose of this study was to document energy exchange and evapotranspiration of dormant and nondormant alfalfa types on a continuous, diurnal basis with energy balance methods.

'Vernal' (dormant), 'CUF 101' (nondormant), and 'Moapa 69' (nondormant) were seeded in April 1989 in adjacent 4.6 m square plots. Border areas were seeded to Vernal with at least 50 m of fetch to all directions but east. The alfalfa was sprinkle irrigated. After the first cutting on 21 August 1989, energy balance instrumentation was installed in the alfalfa plots. Each cultivar plot had one net radiometer and one infrared thermometer. Wind speed, relative humidity, and air temperature were recorded adjacent to the plots at 0.5 m above the canopy. Data were scanned by datalogger every 10 s and averaged or totalled at 30 min intervals.

The (Tc) of Vernal was cooler than Tc of Moapa 69 and CUF 101, indicating that transpirational rates on a unit leaf area basis were greater for Vernal, the dormant type. Further analysis showed Vernal with lower evapotranspiration rates, however, than the nondormant types because Vernal crop height (and therefore leaf area) was less than CUF 101 and Moapa 69 heights. (Crop height is a crucial factor in energy balance calculations and serves as a crude indicator of leaf area index). Vernal canopy resistance averaged 75% of CUF 101 canopy resistance, while cumulative evapotranspiration of Vernal over the measurement period was 91% of CUF 101 evapotranspiration. During the study, winds were primarily easterly instead of the usual westerly, causing fetch to be inadequate most days of the study. Analysis of these data showed that Vernal was more sensitive to advected energy than CUF 101 or Moapa 69, with increased evapotranspiration a main effect. Had fetch been appropriate during the study, differences between Vernal and CUF 101 ET would have been accentuated. If Vernal was the same height as CUF 101, Vernal ET would have been 13% greater than CUF 101 ET. Results of this study and Hattendorf et al. (1990) indicate that alfalfa Tc and ET differ by dormancy type, and ET is affected by rates of crop growth and therefore leaf area. Irrigation scheduling based on Tc and vapor pressure deficit regressions will be inaccurate if a dormant-type relationship is used to schedule a nondormant alfalfa type, and vice versa. Users and developers of crop coefficient curves for irrigation scheduling with alfalfa as the reference crop may need to make adjustments in crop coefficients to account for these differences in alfalfa ET between dormancy types, which are grown in different regions of the country.

Past, Present, and Future of Breeding Bloat-safe Alfalfa in Canada

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Pasture bloat in cattle is caused by the formation of a persistent foam which traps fermentation gases in the reticulo-rumen. It was found that soluble proteins and chloroplast particles in the leaves acted as foaming agents and were the major plant constituents responsible for pasture bloat. Research on breeding a bloat-safe alfalfa was initiated at Saskatoon in 1970. For the first 10 years of this program, major efforts were directed toward determining the causal factors of bloat. A number of theories were formulated, tested and discarded including: Fraction I Protein, Total Soluble Proteins, Foam Volume, and Saponins. The Cell Rupture Theory was proposed based on the demonstration that leaf mesophyll cells of bloat-safe legume species were more resistant to mechanical and microbial rupture than cells of bloat-causing species. It was subsequently found that the initial rates of digestion (IRD) were slower for bloat-safe legumes (sainfoin, birdsfoot trefoil cicer milkvetch) than for bloat-causing legumes (alfalfa, red clover, white clover). From a study of IRD of bloat-causing and bloat-safe legumes it was estimated that a 25-30% reduction in IRD would be required to develop a bloat-safe alfalfa cultivar. Breeding for reduced IRD in alfalfa resulted in a 15% reduction after three cycles of selection. However, a fourth cycle of selection indicated no further progress. The cycle 4 LIRD synthetic seed will be used for uniform alfalfa tests and in pasture trials to assess the reduction in bloat incidence. The LIRD synthetic of alfalfa must demonstrate a significant reduction in bloat incidence before it will be considered for release as a new variety, even on an interim basis.

Bloat-safe traits in bloat-safe legumes were found to include: LIRD, reticular vein structure, thick mesophyll/epidermal cell walls, and condensed tannins. ***Of all these traits, we believe condensed tannins are the key to the development of a bloat-safe alfalfa.*** Data accumulated in the literature indicate that tannins in the foliage would render alfalfa completely bloat-safe. In addition, tannins should provide a "rumen bypass" mechanism to protect alfalfa proteins from excessive breakdown and loss in the first two stomachs. There is also evidence to suggest reduced proteolysis in silage making and pest resistance from high tannin forage. To this end, we have initiated a comprehensive program in flavonoid biotechnology. The program is multidisciplinary and encompasses analytical chemistry, biochemistry, molecular biology, physiology, cytology, rumen microbiology and nutrition, and plant breeding. The major thrust is to use molecular biology techniques to genetically engineer alfalfa foliage to produce palatable condensed tannins. This includes a study of the metabolic pathways and the purification, characterization, and manipulation of flavonoid genes. Condensed tannins from a wide array of legume genera are being purified and analyzed to determine their chemical profile and composition. These profiles are being analyzed for any correlation with known biological/nutritional properties. To achieve these objectives and long-term goals, active collaboration has been established with a number of outside laboratories and institutions.

CAN LONG-TERM ALFALFA FORAGE YIELDS BE PREDICTED FROM SHORT-TERM TRIALS?

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Alfalfa cultivar registration and recommendations are based on forage trials that are harvested for three or four production years. Alfalfa hay producers in western Canada, however, often keep their stands in production for more than four years and seek information regarding the cultivar performance on a long-term basis. Four alfalfa forage yield trials grown in western Canada were harvested for five or six years to determine validity of long-term cultivar recommendations based on three or four years' data.

Error and cultivar covariance matrices varied from trial to trial, but high correlation coefficients ($r > 0.9$) among mean cultivar yields during later production years', suggested that cultivar rankings for year 5 and 6 can be predicted from year 3 and 4 data, respectively. While this is encouraging, more quantitative information about cultivar differences are necessary for economic analyses and recommendations.

The predictability (p) of cultivar differences in their long-term yields was assessed using an efficiency measure based on a multivariate linear model defined as:

$$p = [\text{msepd}(\mathbf{m}, \mathbf{m}, 4)/\text{msepd}(\mathbf{a}, \mathbf{m}, r)]^{1/2} \times 100$$

where, msepd = mean squared error associated with a predicted difference between cultivars, **m** is a vector of constants specifying the long-term yield of interest, **a** is a vector of constants specifying the short-term predictor and r is the number of replications.

Cultivar differences for the fourth, fifth and sixth year yields using the first three years data were not always predictable. Cultivar differences for the four-, five-, and six-year total yields, however, were predictable using equally weighted combinations of three, four, and five years' data, respectively. Four replicates were sufficient for predicting the four- or five-year total yield, but more replicates were needed for predicting the six-year total yield.

This paper starts the process of accumulating information on how long alfalfa cultivar trials must be carried on, how many replications are required, and what predictors should be used in predicting long-term cultivar differences. The usefulness of the statistical measures suggested in this paper need to be tested using more alfalfa yield trials for which long-term yield data are available.

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Expanded Harvest Management of First-Cutting Alfalfa in Kansas

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Management systems have affected stand persistence, forage yield and/or quality of alfalfa (*Medicago sativa* L.) at various locations in the United States: Minnesota (1), Georgia (2), Kansas (3), Iowa (4), South Carolina (5), etc. Harvest schedules for alfalfa in Kansas are occasionally governed by threatening weather, feed shortages, quality demands, insect control, etc. Current information is thus needed regarding the effects of varying management systems on stand persistence and quantity and quality of forage produced. The objectives of our applied research study were: 1) determine the effect of first-cutting management on cumulative forage yield and quality, 2) investigate the impact of this management on stand persistence over two consecutive years of similar treatments and 3) update existing Kansas Extension recommendations regarding early season harvest management.

The 4-site year alfalfa cutting management study was conducted on producer established fields in NE Kansas (rainfed) and Central Kansas (irrigated) and involved eight first-cutting treatments (trts) based on stage of maturity. The soil at the rainfed location (RL) was a Reading silt loam. The experiment was conducted on a 5-year-old stand of 'Kansas Common'. The soil at the irrigated location (IL) was a Crete silt loam and the alfalfa field was a 5-year-old stand of 'Endure'. Early-cut trts (EC) were made at the vegetative and early-bud stage; optimum-cut trts (OC) at late-bud and 10, 25, and 50%-bloom stage; and late-cut trts (LC) were cut at 75-100% bloom and seed pod development. Subsequent cuttings for all treatments occurred at 10%-bloom or when regrowth for the next crop was observed at the crown. Preliminary results indicate significantly lower ($P<.05$) total yield (TY) at the RL for EC-veg. stage while no differences in TY were noted at the IL among EC, OC and LC. Total crude protein (TCP) of EC-veg. stage was lower than OC at the RL, however, TCP of EC-veg. stage was higher than any other trt at the IL. Neutral detergent fiber weighted by yield (YNDF) was highest for EC-veg. stage at RL and lowest for OC at IL. *In Vitro* digestible dry matter weighted by yield (YIVDDM) was highest for EC, late-bud and 10%-bloom stage trts at RL and highest for 10%-bloom stage and lowest for the LC at IL. Relative feed values weighted by yield (YRFV) were higher for the EC-early-bud stage and OC-late-bud stage than other trts at RL. Optimum-cut trts (late-bud and 10%-bloom stage) had higher YRFV than EC and LC at the IL. Percent crown cover (CC) for the OC, with the exception of the late-bud stage, was higher than those of EC at the RL. At the IL, EC-veg. stage had the lowest CC over all trts. Stem counts of OC, with the exception of late-bud stage, and LC at the RL were higher than those of EC; the stem counts of OC were higher than those of EC at the IL. Root total nonstructural carbohydrates (TNC) at OC-10% and 25%-bloom stage were higher than those of EC and LC at the RL while root TNC of the EC-veg. stage was lowest of all trts at the IL. Application of these data for the producer suggest that management strategies involving EC (vegetative stage) would be detrimental to cumulative tonnage and quality (TCP and YNDF). Based on YRFV and YIVDDM data, LC also produced low quality forage. Early-cut trts and OC-late-bud stage caused a reduction in stand persistence over the 2-year study. However, at the IL, EC produced comparable yields and superior quality to OC but again at the cost of stand persistence. Based on our study, Kansas alfalfa producers should avoid stressful early and late first-cutting harvest times which will result in an accelerated loss of stands in old fields.

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Management Practices of Oklahoma Alfalfa Producers

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Alfalfa hay is the second most important crop in Oklahoma. With approximately 400,000 - 500,000 acres harvested annually, alfalfa has a potential value of up to $180 million of income.

In 1988, the first of a 3-part questionnaire was mailed to 4,000 producers, the second and third parts of the questionnaire were mailed to the 520 respondents of part one. Producer responses were 371 for part two and 397 for part three. In 1991, a questionnaire was mailed to 143 producers who had participated in HAYMARKET from 1982 - 1990. Producers completed 85 (59.4%) of the questionnaire. The following figures are a comparison between those surveys.

In 1988, respondents indicated that 93% of the alfalfa was dryland and 7% was irrigated. In 1991, respondents indicated that 79% of their alfalfa was dryland and irrigated increased to 21%. In 1988, 31.4% of the respondents indicated that increased yield was the reason they selected a variety, compared to 1991, where 48.4% indicated increased yield. In 1988, 16% indicated that increased stand life was more important compared to 25.7% in 1991. In 1988, 25% selected a variety based on insect resistance but only 17.9% in 1991 selected a variety for that reason. Disease resistance was also higher in 1988 with 18.2% compared with 9% in 1991.

In 1988, 37% of the alfalfa in Oklahoma was planted to a common variety and 63% was planted to a named variety. In 1991, only 22.5% was planted to a common variety with 77.5% planted to a named variety. In 1988, of the 63% planted to a named variety, 27% was planted to an OSU recommended variety. In 1991, of the 77.5% planted to a named variety, 64.5% was planted to an OSU recommended variety.

In 1988, 41% of the producers treated for insects by visible damage, 14% treated on a scout recommendation, and 7% treated on an applicator recommendation. In 1991, visible damage dropped to 35.7%, scouting reports increased to 29.8%, applicator recommendation decreased to 2.4%, and 32.1% indicated they treated on actual insect counts. In treating for alfalfa weevils, there is virtually no difference in chemical use from 1988 to 1991

In 1991, 38.3% indicated that it is always profitable to keep the fertility adequate on established stands, and 43.2% indicated that it was usually profitable to keep the fertility adequate. 14.8% indicated it was seldom profitable, and 3.7% indicated that it was never profitable to keep fertility adequate. In 1988, 12.7% indicated they apply fertilizer yearly compared with 52.6% that apply fertilizer yearly in 1991. No difference in those that apply every 2 - 3 years, no difference in those that apply every 3-4 years, but in 1988, 45% did not apply fertilizer at any time, and in 1991, that number was reduced to 10.3%.

In 1988, only 12% of the producers soil sampled established stands, while in 1991, that number increased to 37.3%. No difference in 2 - 3, or 3 - 4 years, but in 1988 45% never soil sampled established stands and that number decreased to 20.5% in 1991. In 1988, 54% of the alfalfa hay was not sold, and of the hay that was sold, 54% went to the dairy industry. In 1991, only 8% of the alfalfa hay was not sold, and of the hay that was sold, 66% went to the dairy industry.

Volunteer Levels in Commercial Alfalfa Seed Fields in Eastern Oregon

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Volunteer alfalfa plants contribute to stand contamination and varietal mixing in commercial alfalfa seed fields. The extent and degree of this stand contamination has not been adequately studied in recent years. The Federal Seed Act and state seed certification standards set other variety tolerances and field history requirements to help insure varietal integrity during seed production. Current certification standards in the western states require a field history of a minimum of one year out of alfalfa prior to establishment of a new seed field. The maximum field tolerance for other varieties is 1 in 100 plants (1%) in the northwest states and 1 in 200 plants (0.5%) in California. The objective of this 2 year study was to survey commercial fields, measure volunteer levels, and correlate these levels with field histories.

Thirty newly established seed fields were surveyed during the spring of 1991 and 1992. All fields were sampled after emergence of the planted stand but prior to first cultivation between the rows. At each of five randomly selected sites per field, in row plants were counted using a 4 in. x 4 ft. area marker. At each of 20 randomly selected sites between row volunteer alfalfa populations were measured using the same 4 in. x 4 ft. area marker. Using volunteer populations between rows, an indirect index of volunteer contamination in the row was determined. Average percent volunteer contamination in the planted rows for each of thirty fields is the ratio of mean out of row plants per sample over mean in row plants per sample minus mean out of row plants per sample multiplied by 100. It is assumed that emerging volunteer alfalfa was scattered general over the entire field and that contaminant seedling alfalfa has an equal probability of emerging in the planted row as between the rows. Data on individual field histories and the number of alternate crops in rotation separating alfalfa seed production stands was collected. Average percent volunteer levels of fields with 1, 2 and ≥3 alternate crop histories were statistically compared.

Fig. 1. SURVEY OF VOLUNTEER LEVELS IN ALFALFA SEED FIELDS Malheur County, Oregon - 1991-1992

Field History	Fields Sampled	% Alfalfa Volunteers	
		Range	Mean
1 - Alternate Crop Rotation	7	0.1-10.5	3.5**
2 - Alternate Crop Rotation	12	0 - 2.4	0.8
≥3 - Alternate Crop Rotation	11	0 - 1.3	0.4

** t = 4.057; p = 0.0001

Significantly (P=0.0001) higher levels of volunteer contamination was measured in fields with 1 alternate crop rotation vs ≥2 alternate crop rotations (Fig. 1). The mean volunteer level of 3.5% for the 1 alternate crop rotation, exceeds the other variety certification tolerance in all western states. Some individual fields were found to have volunteer contaminant levels at 5.5% and as high as 10.5% using this survey method. The longer the rotation out of alfalfa seed production, the lower the volunteer percentages in surveyed fields. Fields with two alternate rotation crops and ≥3 alternate rotation crops had average volunteer levels of 0.8% and 0.4% respectively.

The trend in the industry has been to develop, release and produce seed of an ever increasing number commercial varieties. This seed production is occurring to a great extent on a limited number of seed farms. Several divergent factors sometimes compel seed growers to shorten rotations to 1 alternate crop or even none in some cases. In light of this study, this practice may contribute to decreasing levels of varietal purity. This trend should be discussed by alfalfa breeders, seed companies and seed producers.

Alfalfa Seed Production in Manitoba: Genetic and Environmental Factors Affecting Seed Quality

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Alfalfa seed production in the three prairie provinces of Western Canada tripled from 1985 to 1990, but recent overproduction in the U.S. and Canada has caused a leveling off and even a drop in production. Further increases will require the development of new alternatives to traditional production practices. Fifty percent of the cultivars grown on a worldwide basis cannot be increased in Western Canada due to non-dormant fall growth habit and the resulting winter survival limitations. The development of production practices that increase the potential for seed production during the establishment year will allow production from non-dormant types and the potential for increased contract acreage by shortening the time required for seed multiplication.

A preliminary study was established at the Glenlea Research Station (central Manitoba) with 8 alfalfa cultivars covering a range of fall dormancies and planted in a split plot design with seeded and transplanted treatments as the main plots. There was an interaction between cultivars and establishment practice for seed yield indicating that cultivars responded differently depending on plant age. Seed production was limited due to late establishment and pollination problems, but yields in the seeded stands of 200 to 420 kg ha^{-1} indicated that economic seed yields are possible during the establishment year. Low input costs in many regions of Western Canada allow breakeven yields as low as 200 to 300 kg ha^{-1}. Winter survival for non-dormant cultivars was as high as 80% for Grande (dormancy 9), but measurements of spring vigor were closely ranked according to fall dormancy classes.

Seed quality is often a limitation for alfalfa seed production in Western Canada with environmental conditions often contributing to smaller seed sizes and higher levels of hard seed. Although the non-dormant cultivars did generally produced larger sized seed, all cultivars showed high levels of hard seed (68 to 80 %) even though it is generally accepted that non-dormant cultivars produce lower % hard seed. There was a significant establishment practice by cultivar interaction for hard seed content, but the seeded stands which matured later showed a trend toward higher % hard seed. Different overwinter storage treatments also had little influence on hard seed

Measuring seed quality using traditional germination tests has many limitations, but use of a color digital image analysis (DIA) system has the potential to precisely characterize individual seeds and seed lots by seed size and shape and by hue, saturation, and intensity. Preliminary studies have shown that there is sufficient variability within seed lots for each of these factors to distinguish individual seeds. Characterization and subsequent association of traditional seed quality parameters with DIA measurements will be useful for genetic analysis of seed traits and for quality assessment of seed lots.

A three location study similar to the one described has been planted in Manitoba this year with additional seeded stands at Outlook and Melfort, Saskachewan. These experiments will allow detailed analysis of seed quality characteristics over environment, years, establishment practice, and cultivars. Climatic conditions, flowering period and seed set, bee populations, and pollination parameters will be carefully monitered to determine the significant factors influencing seed production.

This research suggests that new alternatives are possible for alfalfa seed production in Western Canada and present experiments will provide information the genetic and environmental factors influencing seed production and seed quality.

Impact of Ease of Tripping on the Self-pollination Rate of Alfalfa

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Breeding for alfalfa (Medicago sativa L.) florets that are easier for honey bees (Apis mellifera L.) to trip has been proposed as a means of increasing the efficiency of pollination and improving seed yield. It is not known if genetically altering ease of tripping affects the proportion of self-pollinated seed produced. This study was conducted to determine if selection for ease of tripping alters the rate of self-pollination.

Easy-to-trip and hard-to-trip populations were developed from 'CUF101' through two cycles of phenotypic recurrent selection, based on evaluations made in the greenhouse with an electronic 'tripping meter' (Teuber, et al. 1988). Plants from the easy- and the hard-to-trip populations, along with plants of the unselected CUF101 parent, were established in separate plots in the field at Davis, CA. Ease of tripping of the populations was measured, self-fertility evaluated, and seed harvested from individual plants in each of two years. Progeny of these plants were grown in the greenhouse, and evaluated for two allozyme loci (Peroxidase-2 and Flourescent esterase-1) using starch gel electrophoresis. Multilocus outcrossing rate (1 - self-pollination rate) estimates were calculated for each population by the maximum likelihood method, using software developed by Ritland (1990).

Ease-of-tripping phenotype of the greenhouse-selected populations was maintained in the field, with the easy-to-trip population significantly easier to trip, and the hard-to-trip population significantly harder to trip than CUF101. Self-fertility did not differ among populations. Self-pollination rate of all populations was lower in 1991 (18.0%) than in 1990 (31.7%), but the Population x Year interaction was not significant. Average self-pollination rate of the easy-to-trip population (27.3%) did not differ significantly from average self-pollination rate of the hard-to-trip population (24.8%) or CUF101 (22.5%). The outcrossing rate averaged 75.1% over all populations.

A previous study demonstrated that easy-to-trip plants had 40.7% higher seed yield than hard-to-trip plants ($P \leq 0.10$) (Knapp and Teuber, 1990). Selection of plants with easy-to-trip florets will likely improve alfalfa seed yield under honey-bee pollination, without altering the proportion of self-pollinated seed.

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Possible Occurrence of a New Biotype of Blue Alfalfa Aphid

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Research on host resistance to blue alfalfa aphid (BAA), Acyrthosiphon kondoi Shinji, has been conducted at Oklahoma State University since 1980. Aphids used in this work have been maintained in greenhouse colonies that are replaced annually with new collections from several locations throughout the state. 'Cuf-101' has been used as a resistant check cultivar since the work began. In flats scored 1 day after termination of infestation in seedling trials,an average of 42% of plants in CUF-101 have been rated as resistant. Another resistant check called Ok 51 was developed in Oklahoma and has had an average of 47% resistant plants. 'Arc' and 'PA-1' have been used as susceptible checks with averages of 2% and 9% resistant plants, respectively. In tests conducted with BAA collected in 1991, the percentages of resistant plants in CUF-101 and OK 51 seem to have declined greatly as may occur with a new biotype. In the studies reported in this paper, we have used the four check cultivars listed above to evaluate this possibility.

The standard test procedure for BAA resistance (1) has been used in conducting evaluations of the check cultivars to ascertain percentages of plants showing resistance to aphids collected in 1991 and 1992. These tests were conducted with three rows of each cultivar randomized per flat and 'Riley' planted in border rows at the ends. Plants were infested 1-2 days after emergence and duration of infestation was 21 days with ratings conducted 7-10 days postinfestation. In a second experiment to determine population growth of BAA on the check cultivars, individual plants arranged in a latin square design in each of eight pots (15 cm dia.) were infested 3 days after emergence. After 9 days, plants were rated on a six point damage scale (1, no damage; 2, slight stunting; 3, moderate stunting; 4, severe stunting and chlorosis; 5, wilting; 6, dead). Development (cotyledon, unifoliolate, or trifoliolate) and numbers of aphids were recorded for each.

Evaluation of check cultivars in flats with BAA collected in 1991 has shown a great reduction in percentages of resistant plants in comparison with results from previous years. The average resistance for CUF-101 has ranged from 12-15%, while that for OK 51 has been 8-10%. Unexpectedly, the resistance of PA-1 has been equivalent to CUF-101. Arc has had 1-3% resistant plants. In the individual plant tests, there were no significant differences in damage ratings (overall mean = 3.4) or plant development (most had formed a unifoliolate leaf). Also, there was no significant difference among cultivars in the mean number of aphids/plant (overall mean = 24).

In conclusion, seedling evaluations in flats indicate that the performance of BAA resistant checks has changed radically in Oklahoma over the last 2 years. Further, expected differences in aphid numbers and damage among resistant and susceptible cultivars in the individual plant test did not occur. These results indicate the possible existence of a new biotype of BAA.

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Selection for Blue Alfalfa Aphid Resistance in Southern New Mexico

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Aphids are major insect pests of alfalfa (Medicago sativa L.). Alfalfa grown in the western United States is often infested with three aphid species: pea (Acyrthosiphon pisum Harris), spotted alfalfa (Therioaphis maculata Buckton), and blue alfalfa (Acyrthosiphon kondoi Shinji) aphids (1). These aphids cause stunting of growth and death to infested plants. Aphid infestations in alfalfa generally occur as dynamic mixtures of more than one aphid species.

In the years before the occurrence of the blue alfalfa aphid (BAA), selection in the New Mexico State University Alfalfa Breeding Program was based on plant survival and overall vigor of mature plants after aphid infestation. When heavy BAA infestations appeared in New Mexico, alfalfa plants became very stunted. Selection following a BAA infestion was based on plant survival and stem length.

Understanding selection effectiveness for survival and stem length is important in developing aphid resistance in alfalfa. To evaluate this selection scheme, three alfalfa populations differing in aphid resistance were subjected to five cycles of recurrent phenotypic selection for survival and mature plant stem length after aphid infestation.

Three alfalfa cultivars (Hi-Phy, Wilson and Malone) were planted during November in a greenhouse floorbed. Initial plant counts were taken at the unifoliolate leaf stage. Alfalfa populations were cutback twice at the ten percent bloom stage. Two weeks after the second cutting, the top five percent of all plants in each population were selected based on survival and stem length, separately intercrossed with honeybees (Apis mellifera L.) and reselected the next year. Populations resulting from five selection cycles were compared with the unselected cultivars for PA, SAA, and BAA aphid resistance.

Results from seven single-species aphid tests using alfalfa seedlings ranged from a significant decrease to a significant increase in percent resistant plants (1). It is reasonable to expect at least small increases in aphid resistance due to selection for survival and stem length after aphid infestation. However, data obtained so far do not provide convincing evidence that the selected populations have a higher percentage of aphid resistant seedlings.

The unselected cultivars and selected populations were retested in the greenhouse floorbed with a mixed aphid population. Positive increases in survival and significant increases in plant height were observed for the selected populations. Gains have been made in the ability of mature plants to survive and continue growth under the mixed aphid infestations. Selection for plant survival and stem length has also increased overall plant vigor in these populations when grown under mixed aphid infestations. However, it is unclear whether aphid resistance as measured by seedling evaluations has been changed.

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Spring Grazing for Managing the Alfalfa Weevil in Grazing Tolerant Alfalfa

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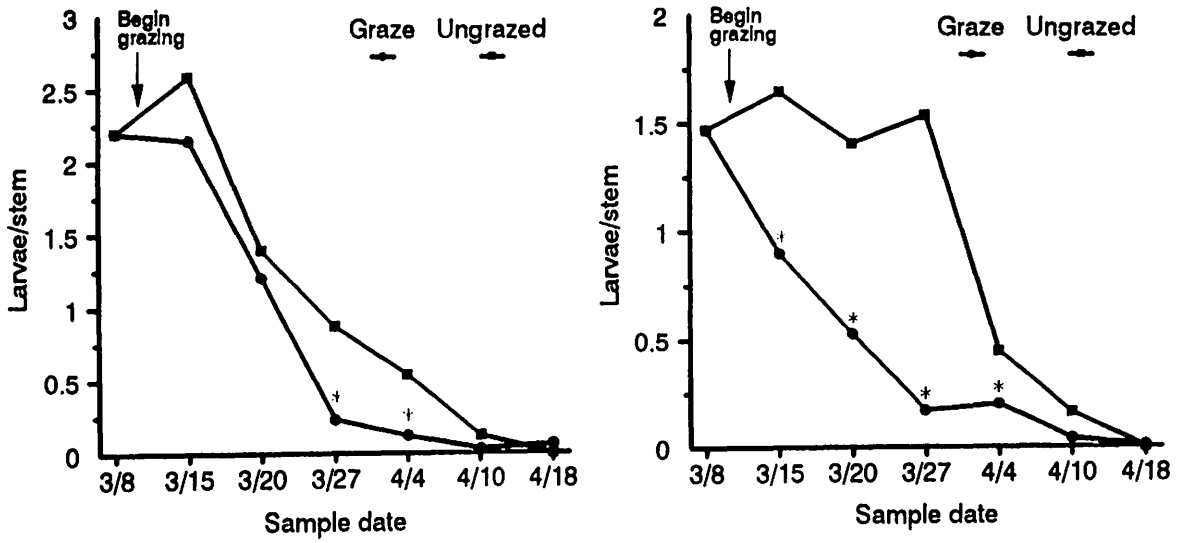
The alfalfa weevil (AW), Hyera postica (Gyllenhal), is a key pest of alfalfa and typically requires at least one insecticide application during the spring to prevent economic damage in the southern USA. Development of grazing tolerant alfalfa permits spring grazing during the time that larvae are active without adversely affecting stand productivity, but grazing restrictions after insecticide use limits spring grazing options. Our objective was to assess whether AW larval populations could be controlled by continuous grazing thus eliminating the need for insecticidal control.

In 1991, 3-year-old plots of 'Apollo' and 'Alfagraze' were used that were arranged in a randomized complete block design with 4 blocks. Plots measuring 2.6 x 5.0 m were divided and a 0.37 m^2 area was caged with a 3 x 6 cm mesh wire cage. In 1992, caged and grazed plots were replicated 5 times in a randomized complete block design in third year stands of both cultivars. Study areas in both years were grazed with cross-bred yearling steers from 9 March to 20 April, 1991 and 3 March to 24 April, 1992. AW larvae were sampled by periodically collecting 20 stems in each subplot and placing samples in Berlese funnels to extract larvae.

Grazing in 1991 significantly reduced AW larval numbers on most sample dates in Alfagraze, but reductions were not significant in Apollo until 27 March (Fig. 1). Reductions in Alfagraze ranged from 45 to 89%. AW larval population were low in 1992 (<0.6 larvae/stem). Grazing generally reduced larval numbers on most dates, but reductions were not statistically significant on any date for either cultivar. Grazing reduced mean total number of larvae collected by 65% in Alfagraze and 32% in Apollo in 1991 and 23% in Alfagraze and 18% in Apollo in 1992. Aphid [primarily pea aphid, Acyrthosiphon pisum (Harris)] numbers were small in 1991, but grazing reduced peak aphid numbers by 94% in Alfagraze and 85% in Apollo in 1992. These preliminary results indicate that insect populations may be effectively managed in alfalfa by continuous grazing in the spring.

Fig. 1. Effect of grazing on AW larval numbers in Apollo (left) and Alfagraze (right) alfalfa in 1991; * indicates significant difference between means on a given sample date ($P = 0.05$; LSD).



Bifenthrin Bioassays of Lygus Bug Populations in Eastern Oregon Alfalfa Seed Fields

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Insect pest resistance to insecticides is a major problem. Few attempts are made to determine the susceptibility of pests to an insecticide when it is first introduced in an area for commercial pest control. The absence of this knowledge makes it impossible to determine if control failures are the result of poor application or resistance. The widespread use of bifenthrin (Capture) insecticide to control Lygus hesperus L. in eastern Oregon alfalfa began in 1990. Bioassays of bifenthrin on adult lygus bug populations were conducted during the spring of 1990 and 1991. Our objective was to use the results of these bioassays to generate baseline LC_{50} values of the insecticide to lygus. The bioassays and baseline LC_{50} data may be useful to monitor for resistance development of this primary alfalfa seed pest.

Small plastic zip lock bags were pretreated with solvent containing concentrations of bifenthrin, bracketing expected residues for effective bioassays as determined from experiments done in 1989. Treatments included 25 μg/bag, 50 μg/bag, 75 μg/bag, 100 μg/bag and untreated control bags. Treatments were replicated 4 times. Bags were stored in a freezer until taken to the field to conduct the bioassays. In the field a small cork and alfalfa trifoliate were placed in each bag. Adult lygus were collected by sweep net and 5 insects were placed in each bag. Bioassay bags were held at ca 20C in a portable incubator (1). After 8 hours in the incubator the lygus bugs were observed in each bag and mortality levels recorded. Ten bioassays were run on field populations of lygus in the spring of 1990, prior to the registration of bifenthrin and its widespread use. Eight additional bioassays were run during the spring of 1991 before any treatments of bifenthrin in its second season of use.

The mean bifenthrin LC_{50} value for the ten 1990 bioassays was 38.9 μg/bag in a range of 26-53 μg/bag. The mean bifenthrin LC_{50} value for the eight 1991 bioassays was slightly higher at 56.1 μg/bag in a range of 25 - 73 μg/bag. Similarly conducted bioassays were run on lygus populations from alfalfa seed fields in Nevada, Washington and California. The eastern Oregon LC_{50} values were quite low when compared to LC_{50} values from other areas.

University and industry data show bifenthrin as a highly effective insecticide to control lygus and other alfalfa seed pests. This baseline data from lygus bifenthrin bioassays combined with field efficacy records may make it possible to forewarn of resistance development with this insect. An early warning of resistance allows for proper and timely resistance management. Pesticide resistance management is a necessary component of an overall alfalfa seed IPM system.

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Searching for resistance to Sitona hispidulus larvae in alfalfa

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Alfalfa plant introductions (150 PIs/year) were seeded near University Park, in rows 3 m long and 61 cm apart in a randomized complete block design replicated 5 times in 1984, 1985, 1987, and 1988. Blocks were subdivided into 4 tiers of 38 rows each for a total of 152 PIs/block (150 PIs and 2 cultivars 'Saranac-AR' and 'WL316'). White clover was seeded in 9.1 m wide alleys between tiers and blocks and along borders to attract adult curculios to the nursery. Weeds were controlled by hand hoeing. Alfalfa was harvested 2-3 times per year. Roots were dug in July the second year following planting, washed, and the percent surface area of the tap root scarred by larvae was estimated. Plants with less than 5% injury to tap roots were sent to Prosser for seed production.

Seed from Prosser was planted in plastic cone containers (4 x 21 cm) in a "Redi-earth" peat vermiculite mix. Four surface sterilized eggs obtained from field collected adults were placed in each cone after plants were 6 weeks old. Pupae were extracted from cones 5 weeks later by flotation in tap water. Pupae were weighed and held for adult emergence. Any larvae recovered were returned to the plants for an additional week. After all insects were recovered as pupae, the plants were replanted and the entire procedure repeated a second time.

PI 183261 and 183263 showed reduced survival of pupae when compared to WL316 (Table 1). PI 183261, 183263, and 183404 had reduced tap root feeding compared to WL316. When the experiment was repeated, percent survival and percent root injury was increased overall for the PIs, but percent survival was usually not significantly lower than for WL316, except for PI 315456-3. However, percent root injury was significantly lower for 5 of the PIs compared to WL316.

Table 1. Percent survival of insect pupae and percent tap root injury.

	First Experiment		Repeat Experiment	
PI	Mean Percent Survival	Mean Percent Injury	Mean Percent Survival	Mean Percent Injury
179702	56.2 ab	5.1 ab	61.2 bc	20.2 a
183060	37.5 ab	3.7 ab	96.7 a	9.2 b
183261	25.0 b	0.7 b	58.5 bc	9.6 b
183263	31.2 b	1.2 b	79.8 ab	8.6 b
183404	43.8 ab	0.4 b	77.1 ab	4.4 b
WL316	76.0 a	8.1 a	47.9 c	19.5 a
231731	23.8 b	2.0 c	28.4 ab	8.3 b
315456-1	59.5 ab	10.7 a	36.9 ab	19.9 a
315456-3	71.4 a	9.9 ab	22.7 b	9.6 ab
315456-5	23.8 b	3.7 bc	28.4 ab	14.4 ab
WL316	47.6 ab	0.9 c	54.0 a	11.7 ab

Means with the same letter are not significantly different at P=0.05.

Influence of alfalfa variety and cutting frequency on potato leafhopper dynamics

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The potato leafhopper, *Empoasca fabae* (Homoptera: Cicadellidae), is generally considered the most serious insect pest of alfalfa in Wisconsin and the upper Midwest. The focus of this report is on the effects of cutting frequency and selected varieties on leafhopper densities in alfalfa. The data are from the first year of a three year, multi-disciplinary project involving the interactions of alfalfa varieties possessing differential levels of resistance to Verticillium wilt, cutting frequency and insect management.

Three alfalfa varieties, Vernal, Apollo II and Apollo Supreme were established in 1990 in plots measuring 9.1 by 13.4 m at Arlington, Wis. Plots were replicated four times in a split-split plot design, with main treatments of cutting schedule (3 or 4 cuts per year), alfalfa varieties (3), and potato leafhopper management (use of currently recommended thresholds and insecticidal control vs. no control). Potato leafhopper adults and nymphs were sampled during 1991 with a D-Vac® ("vacuum net") sampler and converted to numbers per sweep of a sweep net; insect sampling began after first cutting and continued on a weekly basis through August. Leafhopper counts were used to calculate leafhopper days, i.e. the cumulative number of leafhoppers per sweep per day.

Our expectation was that cutting frequency, in particular a schedule of four rather than three cuts prior to September 1, would result in reduced numbers of potato leafhopper nymphs and adults. This expectation was based on the generation time of the leafhopper (3+ weeks under typical southern Wisconsin temperatures) and published reports indicating that severe mortality is suffered by leafhopper eggs and nymphs as a result of the cutting operation. Our analysis indicated that, as expected, adult leafhopper days for the entire growing season were significantly ($P < 0.01$) higher in the three cut than in the four cut plots. However, the magnitude of the difference was not as large as anticipated. Surprisingly, nymph leafhopper days were higher in the four cut than in the three cut plots, with the difference "marginally" significant ($P = 0.07$). Further analysis suggested that nymph survival during cutting may have been much higher than reported in the literature.

We had no basis to expect varieties to have an effect on leafhopper numbers. However, our analysis indicated that variety had a significant ($P < 0.01$) effect on adult and a marginally significant ($P = 0.10$) effect on nymph leafhopper days. For both cutting regimes, adult days were highest in Apollo II and lowest in Vernal; no pattern was evident for nymph days. When adult days were corrected to reflect differences in alfalfa stem densities and heights among varieties, the effect of variety was reduced to marginal significance ($P = 0.07$), and Apollo Supreme replaced Vernal as having the lowest number of adult days.

Relationships of Soil Fertility and Potato Leafhopper Incidence to Alfalfa Yields

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Two of the major factors limiting alfalfa yield, quality and longevity in the mid-Atlantic region are inadequate potassium fertilization and damage from the lack of proper potato leafhopper (Empoasca fabae Harris) control. These two factors are interrelated and thus the fertilization management may affect subsequent pest management decisions. The objectives of this study were to evaluate the effects of P and K fertilization on alfalfa yield and on pest/alfalfa interactions.

The experiment was initiated in June 1987 on a field of 'Cimmaron' alfalfa at the Central Maryland Research and Education Center near Clarksville. The field had been seeded in August 1985. Following the second harvest in 1987, four fertilization treatments were imposed -- 0, 1X (24 kg P + 186 kg K + 2.8 kg B/ha/yr), 2X and 3X rates. To evaluate the impacts of insect damage on yields relative to fertility levels, each fertility treatment was evaluated with and without an insecticide treatment. Plot size was 15.2 x 15.2 m with treatments completely randomized in a factorial design with 4 replications. Yield determinations were from random 0.9 x 6 m areas within each plot.

Incremental increases in P and K fertilization resulted in corresponding increases in adult potato leafhopper populations during a large leafhopper infestation during 1987. Adult leafhopper density at the 0, 1X, 2X and 3X fertility levels were 400, 512, 483 and 570/m^2 without insecticide treatment and 64, 93, 111 and 153/m^2 with insecticide, respectively. Although leafhopper numbers were higher with increasing rates of P and K fertilization, alfalfa injury (as measured by percentage of stems exhibiting burn) decreased markedly with increased fertilization. The only significant yield differences ($p=.01$) in 1987 were between insecticide treated and untreated plots in the third cutting. The dry matter yield loss due to leafhopper damage was 0.68 Mg/ha across all fertility levels.

Significant yield differences were obtained in 1988 for each the 1st ($p=.01$), 2nd ($p=.05$), and 3rd ($p=.01$) cuttings due to insecticide treatments applied in 1987. Since insects were not a problem in 1988, the yield reduction is attributed to a carryover effect of the leafhopper damage in 1987. The total yield reduction for the 5 cuttings affected (cuts 3 and 4 in 1987 and 1, 2 and 3 in 1988) averaged 1.65 Mg/ha across all fertility levels. Significant differences ($p=.01$) in total yield for 1988 were also obtained due to fertilizer treatments. Yields at the 3X fertilizer rate were 1.10 Mg/ha higher than the no fertilizer treatment for both the insecticide and no insecticide treatments.

The only significant yield differences in 1989 were between insecticide treated and untreated plots in the 2nd ($p=.05$), 3rd ($p=.01$), 4th ($p=.05$) and total annual yield ($p=.01$). The yield loss due to leafhopper damage averaged 1.07 Mg/ha. In contrast to 1988, there was no significant response to fertilizer treatments in 1989. Extremely wet growing conditions in 1989 resulted in substantial yield losses early in the season due to bacterial and fungal degradation of plant material prior to harvest, lodged plant material that was not cut by the harvester, and delayed harvests which resulted in a very low 5th cutting. The extremely wet conditions also resulted in variable stand loss as the season progressed. Stand loss was related to drainage patterns rather than treatments imposed. Thus the study was terminated after 1989.

Follow up investigations are currently being conducted to evaluate the influence of soil fertility, grass companion crop (orchardgrass), insecticide and fungicide in reducing pest damage in alfalfa.

Managing Potato Leafhopper on Alfalfa: Paper-Based vs. Computer-Based Systems

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Management of the potato leafhopper (PLH) on alfalfa is required annually on most of the alfalfa in the northeastern and northcentral United States. Strategies to deal with PLH rely largely on insecticide applications. Success of an insecticide focused management program lies not only on insecticide efficacy but also on the economics of the application. Likewise, a no-insecticide recommendation saves the grower application costs as well as opportunity costs. In addition, the negative impacts of the insecticide to the agroecosystem are eliminated. Consequently, we strive to develop management programs which select the most economical control strategy.

Paper-based programs are generally the most common approach to providing pest management information. Traditionally, paper-based programs are very simplistic in their approach to the problem. The majority of programs developed for PLH management focus on insect numbers per unit area and stage of alfalfa growth. These two variables then constitute the basis for a management recommendation. Variables that appear less often in paper-based programs include estimates of hay value and insecticide application costs (both generalized). The more variables used to make a management recommendation the greater the probability that correct action will be taken. Computer-based systems enhance this capability. In addition to handling more variables, they also have the ability to use historic data to forecast events. Likewise, computer-based systems can incorporate interactive insect population and plant growth models to simulate potential pest damage. One such computer-based program developed for managing PLH on alfalfa is PLEX (potato leafhopper expert system). Expert systems are computer-based systems designed to simulate the problem solving of a human expert, thus allowing interpretations where empirical data do not exist. Advantageously, the computer-based system PLEX is site specific, uses real-time information, allows for a variety of different practices, uses current economics, provides for the optimal use of insecticides, and forecasts outcome (yield, % crude protein, root TNC) of the recommendation as part of it consultation. In addition to delivering integrated pest management (IPM), this computer-based program teaches IPM strategies. It also provides information on insect biology, identification, damage, and sampling and can be easily updated.

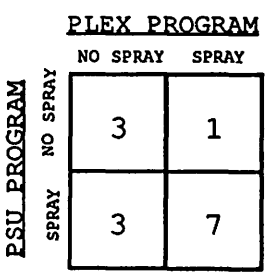

Fig. 1. Performance of PLEX in comparison with the current PSU program.

The performance of PLEX was compared with our paper-based system at various locations in Pennsylvania since 1986. In the 14 field evaluations conducted there were four instances in which the two programs did not agree in their recommendation (Fig. 1). Based on yield and quality analysis of those harvests, PLEX made the correct decision three out of the four times. The average benefit in the PLEX recommendation between these discrepancies was $19.56/A. Large plot to plot variation in yield makes it difficult to measure the amount of yield saved by an insecticide application. Frequently, the projected protein saved drove the decision to apply an insecticide. PLEX accuracy increases with increasing PLH population. PLEX is also being evaluated in Illinois, Kentucky, Maryland, Missouri, Minnesota, and Wisconsin. Results to date support the performance of this computer-based program for managing PLH on alfalfa.

Distribution and Characterization of Virulence Phenotypes Within Populations of Aphanomyces euteiches

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Aphanomyces euteiches Drechs. causes seedling and root rot of alfalfa and several other agronomic crop species. The fungus has been recovered from soils throughout North America and the world. Variability for host range exists among isolates recovered from different hosts as well as for isolates recovered from alfalfa[1]. Alfalfa isolates of A. euteiches display variability in their virulence to different alfalfa populations; for example, "atypical" isolates have been described that are highly virulent to Saranac and to WAPH-1 (the standard resistant cultivar) that are less virulent to certain plant introduction accessions[1].

The objectives of this study were to: add to the base of current knowledge regarding pathogen distribution in U. S. alfalfa growing regions; and, to further characterize alfalfa isolate virulence using the NAAIC standard check cultivars - WAPH-1 (resistant) and Agate (susceptible) and two proprietary breeding lines well adapted to North American growing conditions (Exp 61-A and Exp 61-B). Exp 61-A is a breeding line which has undergone two cycles of selection for resistance to A. euteiches. Exp 61-B represents one cycle of selection for resistance to a mixture of isolates virulent to WAPH-1 out of Exp 61-A. Isolates were tested using previously defined potting, inoculation and scoring methods[1] except that these experiments were conducted at 21°C and zoospores were applied at 800/ml. In addition to individual plant scores (1=healthy to 5=dead), entire pots were given a visual top-growth rating (1=all healthy to 5=>50% stunted, chlorotic plants).

Twenty-seven alfalfa field soils, sampled from 13 states, were assayed using 5-day-old Saranac seedlings as baits. Ten of the 27 soils (from 9 states) were infested with alfalfa-pathogenic isolates. Twenty isolates from each positive soil and 25 isolates previously characterized by Grau, et al. were characterized for virulence phenotype. Average severity index correlated well with whole-pot ratings ($y=1.1x-0.4$, $R=0.93$). Isolates were highly variable for virulence. Four virulence phenotypes could be used to group 173 of the 225 isolates (see Table). Fifty-two isolates demonstrated an array of virulence patterns that if combined would represent a highly variable group.

Isolates recovered from the same field often expressed more than one phenotype, with type 1 isolates predominating in many Midwestern and Eastern soils. There were only two isolates very highly virulent to both Agate and WAPH-1 (A.S.I.≥4.9). Type 3 and 4 isolates were found scattered throughout the U.S. in combination with each other and all other types. It is important to acknowledge the widespread distribution of A. eutieches and its pathogenic diversity so that resistance breeding efforts may be effectively focused.

TABLE. Average Severity Index

	Virulence Phenotype			
Alfalfa Population	1	2	3	4
Agate	4.9	4.9	4.5	4.9
WAPH-1	3.4	5.0	4.9	4.7
Exp 61-A	4.3	4.4	4.3	4.5
Exp 61-B	3.7	3.8	3.3	4.0
No. of isolates	91	2	19	61

Least significant difference 0.05=0.3; CV=4.2%

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Seasonal Occurrence of Alfalfa Foliar Diseases in Iowa

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The seasonality of foliar plant pathogens and their affect on alfalfa yield and quality was studied by establishing field plot experiments in a north-south transect at four research farms in Iowa: Ames, Ankeny, Knoxville, and Chariton. Nine different leaf-spotting fungi and one bacterial leaf pathogen were found to occur at all four locations. These were: *Phoma medicaginis*, *Colletotrichum dematium*, *Xanthomonas campestris*, *Stemphylium botryosum*, *Pseudopeziza medicaginis*, *Leptosphaerulina briosciana*, *Cercospora medicaginis*, *Leptotrochila medicaginis*, *Stagonospora meliloti* and *Uromyces striatus*.

Fungicide concentrations (chlorothalonil) ranging from 0 to 1.26 kg/ha a.i. were applied every 10 days to field plots located in Ankeny and Chariton as a means to create a range of disease intensity values. Regression models relating disease intensity (X) to hay quantity and quality (Y) are presently being developed. Although foliar pathogens significantly reduced quantity of yield (see below) quality was not affected. Yield loss experiments are being repeated in 1992.

Spring black stem, caused by *Phoma medicaginis*, was the predominant foliar pathogen early in the growing season and accounted for 80% or more of the leaf-spot lesions that were present on alfalfa leaves at the time of the first sampling date. The 1991 spring was one of the wettest on record and percent defoliation values caused by spring black stem exceeded 70% in 1991. Yield reductions attributable to spring black stem were 35.1% at Ankeny and 19.7% at Chariton for the first harvest date.

By mid to late June, spring black stem epidemics were largely replaced by common leaf spot (*Pseudopeziza medicaginis*) and *Leptosphaerulina* leaf spot (*Leptosphaerulina briosciana*) with peak periods of *Pseudopeziza* occurring before 18 July. *Cercospora medicaginis* (summer black stem and leafspot) epidemics began in early June and disease severity continued to increase until the third harvest (midAugust). These foliar pathogens reduced hay yield by 31.7% in Ankeny and 2.6% at Chariton at the second harvest and 25.8% and 29.7% at the third harvest, respectively. Other pathogens occurring in low frequencies were *Colletotrichum dematium* and bacterial leafspot (early spring), and *Leptotrochila medicaginis*, *Stagonospora meliloti* and *Uromyces striatus*. These experiments are also being repeated in 1992.

A Biological Disease Forecast System for Fungicidal Control of Sclerotinia Crown and Stem Rot

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Sclerotinia crown and stem rot (SCSR) frequently causes severe damage to forage legumes in Ohio. Apothecia of Sclerotinia trifoliorum emerge in the fall and release ascospores which cause primary infection. The objective of this study was to determine if the fungicide vinclozolin (Ronilan), applied at apothecium emergence, could provide control of SCSR in alfalfa and red clover.

Field plots of Arlington red clover and Armor alfalfa (tentatively classed as 'susceptible') and Hi-phy alfalfa (tentatively classed as 'resistant') were established in pasture sods on Aug. 11, 1988, and Sep. 5, 1989, at Wooster, Ohio, by no-till methods. Fungicide treatments were: 1) No fungicide applied; 2) vinclozolin (1 lb a.i./A) applied at the time of apothecium emergence (Nov. 15, for the 1988 seeding; Nov. 3 for the 1989 seeding); and 3) vinclozolin (1 lb. a.i./A) applied 4 times (mid-Sep., mid-Oct., mid-Nov. and mid-Mar.), to achieve absolute control of SCSR. Fungicides were applied only in the first year establishment period. Data for percent stand, infected plants/plot, and dry matter yield (3 harvests/year) were taken for 2 years following seeding for both trials.

For the 1988 seeding, number of infected plants in April 1989 was significantly reduced by 1 application of vinclozolin at apothecium emergence compared to the unsprayed control for both alfalfa cultivars and red clover. Across all cultivars, the single application of vinclozolin provided approximately 90% of the level of control provided by the 4-spray treatment. In April 1990 virtually no alfalfa plants showed symptoms of SCSR, while numerous red clover plants continued to die, indicating that red clover, but not alfalfa, was susceptible after the first year. Vinclozolin applied at apothecium emergence increased dry matter yields by 42, 30, and 83% over the unsprayed control for Armor and Hi-phy alfalfa and Arlington red clover, respectively, in the first production year. Dry matter yields in plots treated with 4 applications of vinclozolin had significantly greater dry matter yields than plots receiving a single fungicide application, with corresponding increases over the untreated control of 73, 59, and 123%. There was no significant effect of any fungicide treatment on total dry matter yield in the second production year.

In the 1989 seeding numerous infected plants were found in mid-March in the unsprayed control plots. However, infected plant counts were not made until April 24, by which time plants that showed symptoms earlier had rotted and disappeared. Consequently, the greatest number of infected plants were found in plots receiving one application of vinclozolin at apothecium emergence. Percent stand estimates at this time were 6, 63, and 72% across all cultivars for the unsprayed control, 1 application, and 4 applications of vinclozolin, respectively. This estimate more accurately reflected damage from SCSR than number of infected plants/plot. Vinclozolin applied at apothecium emergence increased dry matter yields by 39, 35, and 65% over the unsprayed control in the first production year, and by 28, 38, and 58% in the second production year for Armor and Hi-phy alfalfa and Arlington red clover, respectively. No significant increase in total seasonal yield was obtained with 4 applications of vinclozolin in comparison to the single application treatment.

Results indicate that vinclozolin applied at apothecium emergence in the fall can provide control of SCSR in alfalfa and red clover and substantially increase forage yields.

Disease x weed interactions in irrigated alfalfa

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Alfalfa is grown on more acreage in Wyoming than any other crop. Alfalfa producers are confronted with a number of crop pests including diseases and weeds especially when grown under irrigation. Data on disease and weed interactions is extremely limited (1). Therefore the objective of this research was to evaluate the impact of a disease susceptible and resistant cultivar, seeding rate and pesticide treatment on alfalfa, weeds and disease over a 5 year period.

The trial was established May 1986 near Dayton, WY on a clay loam soil. The average annual precipitation at the site was 12 inches with additional moisture (~ 12 inches) provided by flood irrigation. The site was naturally infested with annual bluegrass (Poa annua L.), dandelion (Taraxacum officinale Weber in Wiggers), common lambsquarters (Chenopodium album L.), field pennycress (Thlaspi arvense L.), Shepherd's purse (Capsella bursa-pastoris (L.) Medicus), quackgrass (Elytrigia repens (L.) Nevski), Phytophthora megasperma Drechs. f. sp. medicaginis Kuan & Erwin, Verticillium albo-atrum Reinke & Berth. and Ditylenchus dipsaci (Kuhn) Filipjev. The experimental design was a randomized complete block with a factorial arrangement and four replications. Experimental units were 1.5 by 4.6 m and consisted of 10 alfalfa rows with 15.2 cm between rows. Forage yield (alfalfa and weed), plant and weed populations, and disease rating were determined during the 5-year period. A total of 10 harvests were taken off the plots before the study was terminated.

Alfalfa yield and seeding rate were positively correlated, whereas weed yield and seeding rate were negatively correlated. The lowest alfalfa yields occurred in the non-pesticide control plots. The yield from plots receiving both herbicide and fungicide/nematicide were significantly higher than either chemical along. There was a significant decline in alfalfa stand overtime regardless of treatment. As disease increased, weeds became more prevalent in the non-pesticide control.

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Growth Chamber Methods to Evaluate Resistance of Forage Legumes and Grasses to Pratylenchus penetrans

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The root-lesion nematode (Pratylenchus penetrans) can reduce the establishment of forage legumes, including alfalfa, in hay production fields, as well as in pastures. Alfalfa is frequently seeded in mixtures with cool-season grasses and is interseeded into grass swards during pasture renovation. Therefore, it is important to consider the host suitability and preference of root-lesion nematodes for forages that are grown in combination with alfalfa.

Six forage legumes and grasses were compared for resistance to P. penetrans. Baker and MNGRN-16 alfalfas, kura clover, sainfoin, quackgrass, and perennial ryegrass were grown in individual plastic tubes (72 tubes per entry) in a growth chamber (25° C). One week after planting, 36 tubes per entry were inoculated with 200 P. penetrans. After 15 weeks, shoot and root dry weights of all entries were reduced by P. penetrans ($P<0.01$). Baker alfalfa, sainfoin and kura clover had about 6,000 nematodes/root system compared to 4,000 for quackgrass, 2,600 for MNGRN-16 alfalfa, and 800 for perennial ryegrass.

In a second test, eleven forage legumes and grasses were evaluated for resistance to P. penetrans as previously described except that 8 tubes/entry received 0, 200, 400 or 800 P. penetrans/tube, two weeks after planting. Fifteen weeks later, shoot and fibrous root dry weights were reduced by P. penetrans ($P<0.01$). Nematode reproductive factors (final nematode population/initial nematode population) were calculated for each entry inoculated with 200 nematodes. The reproductive factors were: 'Norcen' birdsfoot trefoil (58), common kura clover (39), 'Eski' sainfoin (27), 'Baker' alfalfa (23), MNGRN-4 alfalfa (15), MNGRN-14 alfalfa (14), MNGRN-16 alfalfa (6), 'Palaton' (3) and 'Rise' (5) reed canarygrass, and 'NK-200' perennial ryegrass (5). Reproductive factors and fibrous root weights decreased as inoculum levels increased.

We concluded that : (1) The forage species differed in resistance to P. penetrans. (2) Based on numbers of nematodes and root and shoot weights, MNGRN-16 alfalfa was the most resistant forage evaluated. MNGRN-16 was developed at St. Paul by three cycles of recurrent selection for resistance to the root-lesion nematode. (3) Inoculum level is critical in nematode resistance evaluations. (4) This long-term growth chamber test can be effectively used to evaluate forage species for resistance to P. penetrans.

Reaction of Nine Alfalfa Entries to Mixed Populations of the Alfalfa Stem and Chrysanthemum Foliar Nematodes

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The chrysanthemum foliar nematode (CFN), Aphelenchoides ritzema-bosi, has been reported to occur in association with the alfalfa stem nematode (ASN), Ditylenchus dipsaci in alfalfa (1,2). In a recent survey, CFN was recovered from 92% of 58 samples collected in California, Colorado, Idaho, Montana, Oregon, Washington, and Wyoming which exhibited typical ASN symptoms (swollen stem buds, stem necrosis, white flagging, and leaf distortion) (3,4). CFN constituted 23% of the total combined nematode (CFN and ASN) population from all samples. CFN percent composition in individual samples ranged from 0 to 94%. Due to the frequent occurrence of both nematodes in ASN-symptomed tissue, our present study was initiated to determine the reaction of alfalfa cultivars to a mixed population of CFN and ASN.

Nine alfalfa cultivars were tested. Six cultivars were field selected for ASN resistance; Vernema, Falcon, Cougar, FSRC-IH-171, Caliverde 65, and Lahontan. One cultivar (W2S2) was developed with monoxenically cultured ASN inoculum. Two were susceptible checks (Ranger and Moapa 69). Seedlings and 6-week-old plants were inoculated with mixed populations of ASN and CFN collected from ASN-infected stem bud tissue. After 4 weeks, plants were rated for symptoms of ASN and CFN infection and nematodes were extracted from ASN-symptomed tissue using the Baermann funnel extraction procedure. Nematodes were identified at 100 to 400x.

Resistance to the CFN was not detected in seedlings due to limited infection and/or reproduction. The CFN was recovered from only 3 of the 9 entries in each of the seedling experiments. Seedling mortality occurred in all entries and increased with the number of nematodes applied.

Both ASN and CFN were recovered from all entries in 6-week-old plant experiments. Disease severity ratings for stem/leaf symptoms were significantly higher in Experiment 1, which had an inoculum concentration of 56% CFN, than in Experiment 2, with a concentration of 6.2% CFN. However, there was no significant difference between entries relative to the percent CFN recovered in either experiment. There was also no differential resistance to CFN between field selected entries and W2S2, selected with monoxenically cultured ASN inoculum, or between entries selected from coastal California where the percent CFN in tissue may be relatively high, and other areas where the percent CFN is lower. In both 6-week-old plant experiments, there was less total nematodes/g of dry tissue and less CFN/g of dry tissue in ASN-resistant entries than in susceptible entries. This difference was highly significant ($P < 0.05$) in Experiment 2. This implies that field selection for ASN may have increased resistance to both the ASN and the CFN.

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Development of a Molecular Marker Linkage Map of Diploid Alfalfa

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Methods of molecular biology have been developed for detecting genetic markers which are based on differences in nucleotide sequences of homologous DNA fragments. The most widely used DNA markers are restriction fragment length polymorphisms (RFLPs) (2) and random amplified polymorphic DNAs (RAPDs) (3). These molecular markers have many potential applications in crop improvement (2). For some of these applications, such as those involving mapping and manipulation of genes in a breeding program, it is necessary to determine the linkage arrangement of markers in the genome. Knowledge of linkage arrangement allows the researcher to choose markers such that all regions of the genome can be analyzed.

Due to the complex nature of segregation analysis in tetraploids, we chose to initially develop a linkage map using diploid alfalfa. The population was derived by crossing two parents from different populations of CADL (1). One F1 plant was crossed to one of the parents to derive a backcross (BC) population. The recurrent parent, F1 and 88 BC plants were analyzed for RFLPs using genomic DNA and cDNA as probes on Southern blots, and for RAPDs using arbitrary 10 base oligonucleotides as primers with the polymerase chain reaction. These probes and primers had been prescreened to select those that detected polymorphism between the parents.

The use of heterozygous parent plants resulted in several observed segregation patterns for marker loci. RAPD loci segregated 1:1 (80 loci) or 3:1 (21 loci). RFLP loci segregated 1:1 (55 loci), 3:1 (3 loci), 1:2:1 (13 loci) or 1:1:1:1 (13 loci). In order to construct a single linkage map including all segregating loci, we used a recently developed computer program, G-Mendel 2.0. This program will determine the linkage groups, the most likely order of loci within linkage groups, and the map distances between adjacent loci for several different types of segregation data in a single population. The amount of genetic information for linkage analysis between loci with different segregation patterns varies greatly, and the initial map based on the entire data set (167 loci) tended to cluster loci which were the most informative with each other. To increase the overall amount of linkage information in the analysis, we constructed two maps using subsets of loci which were completely informative within each subset. The larger of the two maps is 629 cM and includes 32 RFLP and 45 RAPD loci. We are now working to combine the two maps using marker loci that are informative in both data subsets.

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Association of RFLP-Based Genetic Distances Among 2x and 4x Alfalfa Lines with Performance of their Single-Cross Families

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Tetraploid alfalfa populations containing a maximum of two alleles per locus (diallelic-duplex) were created by chromosome doubling diploid hybrids (1). Diallelic-duplex lines and their diploid progenitors were used as parents to generate 4X4 diallels at the 2x and 4x ploidy levels. Based on the established correlation between maximum heterozygosity and heterosis in autotetraploid alfalfa (2), highest yielding populations should be obtained from hybridizing individuals with the highest number of allele differences. Compared to the time and resources consumed by progeny testing large numbers of genotypes, the use of molecular markers provides an opportunity for efficiently assessing the level of genetic diversity among parental lines within heterogeneous populations (3). The feasibility of using molecular markers for predicting heterosis in alfalfa hybrids was tested by comparing the levels of polymorphism for DNA restriction fragment lengths among parents in the 4X4 diallels with the yield performance of their resulting single-cross (SC) progenies.

Performance of SCs was evaluated in replicated field trials over a two year period. As has been observed in other studies in alfalfa (4), significant differences in yield were detected among SC progenies but rankings were inconsistent across ploidy levels. Highest and lowest yields were obtained from crosses involving the same parents at both ploidy levels, however. Diploid and tetraploid parental lines were evaluated for restriction fragment length polymorphisms (RFLPs) using 53 recombinant DNA clones. Fragment data were used to calculate RFLP-based genetic distances according to the methodology of Nei and Li (4) and values agreed with what was expected based on pedigree analysis.

In order to evaluate the relationship between genetic diversity and heterosis, regression analyses were conducted on yield data obtained from SC families and their respective RFLP-based genetic distance values. In results from the analysis at the 2x level, a very low association between genetic distance and performance was detected indicating that RFLP-based genetic distance measurements did not accurately predict performance of SCs of these diploid lines. However, a high correlation between performance of 4x SCs and RFLP-based genetic distances was detected and this association ($r=0.84$) was statistically significant at the 5% level. These preliminary results support the feasibility of developing a system for effectively predicting hybrid performance of crosses based on molecular marker differences among alfalfa lines at the tetraploid level.

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Development and Application of an RFLP Linkage Map for Diploid Alfalfa

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We are developing a linkage map in diploid alfalfa. The two parents were derived from the W2xiso population and from PI440501 (*Medicago sativa* ssp. *coerulea*). A single F1 from this cross was selfed to produce a segregating F2 population of 86 individuals. Clones from a cDNA library were screened on parental and F1 survey filters; single copy clones showing clear segregation patterns, either 1:2:1 or 3:1, were then screened on the F2 population. Data was analyzed using the MAPMAKER computer package and a linkage map was produced. The current map (as of 6/11/92) has 104 cDNA markers covering 458.8cM in ten linkage groups. Clusters of cDNA clones were seen in several locations. We plan to add genomic and RAPD markers to the map in the near future to further cover the genome. Approximately 50% of the clones showed segregation distortion, almost always in the direction of excess heterozygotes. This may provide circumstantial evidence for the necessity of maximum heterozygosity in alfalfa plants. We are now beginning a project to use the map in identifying chromosomal regions associated with acid-soil (aluminum) tolerance.

Use of Molecular Markers to Identify Hybrids of Self-Incompatible Alfalfa

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Ten to fifteen percent increase in forage production can be obtained in hybrid alfalfa, but production of hybrids using available male sterility systems is inefficient due to nonpreference of bees for the pollen-deficient male steriles. In our research alfalfa exhibiting a high level of self-incompatibility (SI), acceptable pollen viability, and female fertility are being selected as parents for producing hybrid alfalfa. Ten elite self-incompatible alfalfa plants selected from two broad-based multiple pest-resistant lines have been clonally propagated and are being evaluated. Due to the lack of usable morphological or isozyme traits in this elite germplasm, restriction fragment length polymorphism (RFLP) analysis is being used to determine hybridity in crosses of these clones. Southern blots of *Eco*RI, *Eco*RV, *Hind*III, and *Bam*HI digested genomic DNA were hybridized with random-primed labelled probes from random alfalfa seedling cDNA clones amplified using the polymerase chain reaction (PCR) . More than 85% of the inserts hybridized to low copy number sequences, and all 12 of the probes tested have detected RFLPs in these different clones. *Eco*RV and *Eco*RI digests revealed the highest number of polymorphisms out of 24 probe/enzyme combinations scored, but *Eco*RI RFLPs were easier to score due to a lower average size. Cluster analysis was performed on simple matching coefficients for all visible bands scored to determine the degree of genetic similarity of the different SI clones. Three clones, not all from the same parent population had nearly identical banding patterns (93%), whereas the others had banding patterns which were less than 75% similar to each other and to the other cluster of closely related individuals.

Three clones which were not closely related based on cluster analysis were chosen as parents to assess the feasibility of using RFLP markers to detect hybrids. Two of the clones were from the population (BMP8) and the other was from the unrelated population (W10). The clones were crossed using a diallel crossing scheme and the progenies were evaluated using *Eco*RI and *Hind*III and four probes (U5, U25, U26, and U27). The self progeny were evaluated to identify bands which were present in more than one copy since such bands would appear in 83% of the hybrids.

Interpretation of the initial results are complicated due to the presence of extra bands which were not present in the parents. The extra bands were not probe or enzyme specific, and the extra bands were usually observed for more than one probe/enzyme combination. Most of the extra bands corresponded to those observed in the other seven SI clones. Self progeny from one of the clones (BMP8-45) was very uniform suggesting that the clone was free of plants from the other clones. Analysis of this clone (BMP8-45) crossed with a self-incompatible from an unrelated alfalfa population (W10-710) revealed that 18% were selfes and at least 48% were hybrids. The actual percent of hybrid seed produced on BMP8-45 is probably much higher but because of the presence of extra bands not belonging to W10-710, the identity of the pollen donor is uncertain.

Upon further evaluation of individual plants of the clonal material, we discovered that many of the plants within the clones were no longer identical genetically. We are using the cluster analysis to identify which plants are the original SI plants and once the contaminating plants are discarded, we will repeat the diallel crossing scheme.

Despite our problem with maintaining pure plant material, we have been successful in identifying probe/enzyme combinations which can identify genetically individual tetraploid alfalfa plants. This technology can be utilized to fingerprint self-incompatible alfalfa germplasm for the identification of hybrid alfalfa.

Associating Molecular Markers With Disease Resistance

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Molecular markers provide an opportunity to develop new methods of improving plant populations through the incorporation of marker-based selection into conventional breeding programs. However, the current methods of mapping positions of markers onto genetic maps are inadequate for placing markers proximate to genes conditioning traits of interest in tetraploid alfalfa populations. The difficulty stems from the complex nature of genetic control of most traits of interest in alfalfa, and the large populations needed to obtain adequate numbers of individuals displaying the trait of interest in a collection of progeny from a pair of parents. We are developing a method of associating random amplified polymorphic DNA (RAPD[2]) markers with disease resistance in agriculturally useful alfalfa genotypes. Initial studies are based on resistance to *Peronospora trifoliorum* deBary (downy mildew).

Seedlings of UC123 and UC143 alfalfa were tested and selected for susceptibility and resistance, respectively, to *P. trifoliorum* isolate I-8. UC143 was derived from UC123 through selection for resistance to isolate I-8 (1). The RAPD procedure (2) was carried out on 48 plants from each of these populations. The frequencies of occurrence of individual RAPD fragments, and combinations of two and three fragments, were tabulated for each population, and statistically compared. Significant differences between populations were sought as an indication of fragments associated with resistance. Ultimately, such fragments will prove useful in selecting for disease resistance.

Combinations of fragments significantly more frequent in UC143 than in UC123 were found (Table 1), suggesting an association with resistance. However, these combinations of fragments were not present in all resistant plants. Similarly, combinations of fragments significantly more frequent in UC123 than in UC143 were found (Table 1). The associations again were not absolute, indicating that recombination occurred between at least one of the fragments and the genes involved in disease reaction. These results indicate that the method under development is feasible and will yield useful markers with further refinement.

Table 1. Frequencies of occurrence of three-way combinations of random amplified polymorphic DNA fragments in alfalfa populations susceptible (UC123) or resistant (UC143) to *Peronospora trifoliorum* isolate I-8.

Fragments			Percentage Frequency in UC123	UC143	Chi-square	Probability
M01.1	M01.2	M02.2	18.7%	52.9%	11.0	<0.01
M01.1	M01.2	M06.4	6.0	48.8	48.9	<0.01
M01.1	M01.2	M07.3	5.5	46.2	24.7	<0.01
M01.1	M01.2	M04.6	0.0	42.1	35.1	<0.01
M01.1	M01.4	M04.2	64.7	29.4	9.5	<0.01
M01.1	M04.2	M06.1	70.0	28.1	11.0	<0.01

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Expression of alcohol dehydrogenase enhances flooding tolerance in transgenic alfalfa

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The objective of our work is to enhance the persistence of alfalfa in the Northeastern region of North America by enhancing its tolerance of environmental stresses, including freezing, ice-encasement, and flooding. Our recent approach has been to introduce genes which have been implicated in the tolerance of one or more of these stresses using the techniques of genetic engineering and thereby modify the pattern or level of accumulation of the protein (enzyme). We subsequently determine the effect of this altered enzyme activity on the transgenic plant's tolerance of stress. Two genes which are currently under study are superoxide dismutase (SOD) and alcohol dehydrogenase (ADH). SOD has been widely implicated in the tolerance of oxidative stress which occurs during periods of freezing, drought, et cetra. ADH, in conjunction with pyruvate decarboxylase, catalyzes the anaerobic conversion of pyruvate to ethanol. This allows the fermentative respiration of sucrose if oxygen supply limits respiration as occurs during soil waterlogging or ice-encasement. Low ADH activity has been associated with low rates of gycolysis and low rates of energy production during periods of anoxia. Inhibition of ADH with the inhibitor pyrazole increases injury induced by root anoxia.

To determine if enhanced or altered ADH activity would influence alfalfa's tolerance of soil waterlogging, a number of transgenic plants were constructed in cooperation with Plant Genetic Systems, Gent, Belgium. A genomic *adh* gene from Arabidopsis thaliana isolated by R. Dolferus was modified by site specific mutagenesis to allow the Arabidopsis promoter to be removed and replaced with the TR2' promoter from Agrobacterium which was believed would provide root specific expresion and auxin inducibilty to the *adh* gene. This modified gene was introduced into a binary transformation vector, pADH3, which contained the left and right border sequences of the Agrobacterium T-DNA, and the *bar* gene coding for resistance to the herbicide phosphinothricin as a selectable marker.

Petiole explants from the alfalfa line RA_3 were transformed with Agrobacterium C58C1Rif pMP90 pADH3 and cultured using standard procedures in the presence of phosphinothricin. Callus was induced to form somatic embryos by treatment with 2,4-D and several putative transformants were regenerated. Transformation was confirmed by resistance to phosphinothricin, accumulation of PAT activity (product of the *bar* gene), the presence of an additional group of ADH isoenzymes in callus and root tissue extracts, and by a PCR probe for the TR2'-adh construct. In one of the transgenic plants the resistance to phosphinothricin was present in 67 cross-pollinated progeny in a 35:32 (tolerant:susceptible) ratio indicating a one gene insertion.

Ten transgenic plants were clonally propagated by cuttings that were evaluated for flooding tolerance in 3 separate tests of 15 plants per test. The experimental design was a split plot in time arrangement with systematic controls in each block. The plants were submersed in deareated tap water for 21 days. Survival and regrowth was estimated for 3 cycles of regrowth after removal from flooding. One transformant, RA_3-adh3-3, was significantly more tolerant of flooding than the non-transgenic control and the other transgenic plants. This plant was also more tolerant of ice-encasement, but the increased tolerance does not seem to be due to increased total ADH activity. The inheritance of this flooding tolerance and the *adh* transgene is under investigation.

Variability for Stem Anatomy Among Entries in the Perennial Medicago Core Collection

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Lignin concentration is closely related to forage quality. Alfalfa stem anatomy also may be related to forage quality. Kephart et al. (2) reported that lines selected for high digestibility (i.e. low lignin) were shorter, lower yielding, and had more vascular bundles in the stems. They suggested that the greater leaf-stem ratios in highly digestible lines required more vascular support. Akin et al. (1) found that the lignified ring and vascular tissues in the stem were the main contributors to textural strength of the forage. Stem anatomy could also affect aspects of forage utilization by ruminants. Lenssen et al. (3) suggested that the higher digestibility of some diploid glandular-haired species was due to a larger cortex and smaller pith than in hay-type alfalfas. Physical properties of stems also could affect the ease of chewing and retention time in the rumen. The goal of this research was to evaluate differences in stem anatomy among plant introductions of perennial Medicago species.

We evaluated the stem anatomy of 221 alfalfa plant introductions, designated as the Alfalfa Core Collection. The Core Collection represents most of the available genetic variability in the species. In addition we included four checks: Vernal, Saranac, WL 322 HQ, and KS186GH9SP6SAI. The study was planted at Rosemount, MN in a randomized complete block with five replicates for a total of 1125 plots. Six to 10 stems per plot were sampled from the first regrowth by collecting either the internode above the first flowering node, or the third internode from the apex if no flowers were present. The samples were fresh frozen. Razor blade cross sections were prepared from each stem in a plot. Cross sections were immediately stained with a drop of 1% phloroglucinol solution, then observed under a microscope at 50X magnification. Stem cross sections were evaluated for six stem anatomy traits: number, size, and arrangement of vascular bundles; ratio of pith to stem diameter; lignified tissue type; and percent lignification. Thirty-nine entries were chosen as representative of the observed variability.

Significant differences were observed among entries for all traits. Ratio of pith to stem diameter was the least variable while percent lignin was the most variable. Percent lignin and lignified tissue type were the most highly correlated traits ($r = 0.635$). Entries showed most combinations of traits; for example, some entries had large bundles and high lignin, other entries had large bundles and low lignin. Variability between entries in the Alfalfa Core Collection for stem anatomy traits indicates that plant introductions may be a valuable resource for improving stem anatomy traits. Low correlations between traits indicate that predicting one trait from another may be difficult. The practical value of stem anatomy traits should become more obvious when they are related to forage feeding value.

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*This research was a B.S. Senior Project by the senior author.

Tissue Culture Screening Procedure to Develop Alfalfa Germplasms Tolerant to Acid, Al-Rich Soils

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Alfalfa is a very sensitive species to acid soils with toxic levels of aluminum (Al). The development of alfalfa germplasms tolerant to acid, Al-rich soils could extend the use of alfalfa to marginal lands, thus providing high quality forage at lower cost. The use of tissue culture to select plants tolerant to heavy metal toxicity has been shown by several authors. Parrott & Bouton (1990), studying Al tolerance of two alfalfa germplasms as expressed in tissue culture, concluded that cell culture can be used to screen for acid or Al tolerance. The objective of this experiment was to test if selection for Al tolerance, based on a tissue culture assay, was effective in producing germplasm with better performance in acid soil under greenhouse conditions. The germplasms were selected using the same procedure adapted by Parrott and Bouton (1990). Initially calli were induced from petioles of 100 randomly selected plants from the experimental germplasm 'Georgia TE' in a modified Blaydes medium for 6 weeks. After that, the calli were divided into equally sized pieces and transferred to 2 different media; one that was modified by lowering the pH to 4.0 and lowering levels of calcium and phosphorus to simulate acid soils, and one with the same modifications, plus toxic levels of Al. The calli were transferred to each respective medium every 2 weeks, totaling 8 weeks of growth. After this time, the plants with the best ratio obtained by dividing their net callus weight on Al medium by their net callus weight on the medium without Al, were selected and intercrossed to form a new germplasm. This new germplasm was again selected in tissue culture and intercrossed to form a germplasm called 'High Ratio' (HR). Similarly, the ten plants with the best net callus weight on the Al medium were selected and crossed for 2 generations to form a germplasm called 'High Net' (HN). The resulting germplasms were evaluated in the greenhouse using 720 ml styrofoam cups filled with 930 g of soil. The soil was a Cecil sandy loam with the following characteristics: pH_{water} = 4.70, Al_{KCl} = 0.29 me /100 g (Al saturation = 41%), calcium = 0.28 me /100 g, magnesium = 0.07 me /100 g of soil, phosphorus = 8 kg ha^{-1} and potassium = 54 kg ha^{-1}. The soil treatments were: a) cups completely filled with limed and fertilized soil (LFS); b) cups completely filled with unlimed and unfertilized soil (UL) and c) cups first filled with 720 g of unlimed and unfertilized soil and then topped with 200 g of limed and fertilized soil (PLFS). The germplasms tested were: 'AT', an acid tolerant germplasm derived from three cycles of recurrent selection (Bouton and Radcliffe, 1989), and which has served as an check for acid tolerance, Georgia TE, and the two 2 germplasms, HR and HN. The plants were grown for 8 weeks at which time the cups were marked at 11.25 cm from the base and cut into 2 portions, the top, including crown and the top 3.5 cm of roots and bottom portion with the remaining roots.

The soil used greatly reduced yield of all germplasms. Top yield was not affected either by the LFS or PLFS, but HR and HN tended to yield more than the other germplasms. HR yielded more roots than all others in both LFS and PLFS. In the UL, HR was the best germplasm, yielding significantly more than any other germplasm. Although these results need to be confirmed under field conditions, selection for tolerance to acid, Al-rich soils by screening parents based on their performance in tissue culture seems feasible and could be coupled to a whole plant selection screening procedure to produce tolerant germplasm.

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Allozyme Characterization of National Alfalfa (Medicago sativa L.) Cultivars From the Peoples Republic of China

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During 1990 and 1991 a total of 94 M. sativa germplasm pools from the Peoples Republic of China (PRC) were introduced to the United States of America (USA). Very little information is available from either Chinese or American literature about the characteristics of this germplasm. The objectives of this research are to characterize the allozyme, morphological, and agronomic variation among and within PRC alfalfa germplasm resources and to compare that variation with the variation present in alfalfa germplasm currently used in the USA.

This report is a preliminary analysis of the 23 officially approved (National) Chinese cultivars. Nine USA cultivars (Norseman, Vernal, Ranger, Saranac, DuPuits, Lahontan, Mesilla, Moapa 69, CUF101) were also included as standards. Forty-five individuals from each of the accessions were planted as a randomized complete block design with three replicates in the greenhouse at Davis, CA. Allozyme allele frequencies were determined for leucine amino peptidase locus two (LAP 2), peroxidase locus two (PER 2), and fluorescent esterase locus one (FEST 1) using the boric acid system of Quiros (1981) and the staining recipes of Soltis et al. (1983). Allelic frequencies at each of the allozyme loci were used to determine Nei's genetic distances and "heterozygosity." Genetic distances were subjected to a paired group method of cluster analysis. Software for computing Nei's distances and the cluster analysis was obtained from Dr. Kermit Ritland (Dept. of Botany, University of Toronto, Toronto, Ontario M5S 3B2, CANADA).

PRC and USA germplasm differed in both the number of alleles present and the allele frequency. As many as four alleles ($x = 3.3$) were present in PRC germplasm compared to a maximum of three alleles ($x = 1.9$) in the USA check cultivars at the LAP 2 locus. Two alleles were present at the PER 2 locus and three alleles were present at the FEST 1 locus in both PRC and USA cultivars. The greater frequency of multiple alleles in PRC cultivars contributed to higher estimates of heterozygosity relative to USA cultivars. Genetic diversity among PRC cultivars was much less than among the USA check cultivars suggesting a narrow genetic base for PRC cultivars. However, the combined diversity of the cultivars we sampled from both countries is greater than the diversity represented from either country alone. Useful genetic variation in both the PRC and the USA may be increased by introduction and utilization of the other countries genetic resources. Evaluation of allozyme , morphological, and agronomic characteristics of all 95 introductions is continuing.

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Lucerne Improvement in New South Wales, Australia.

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NSW Agriculture initiated a breeding program for lucerne at Yanco in 1978 following the introduction of the spotted and blue-green alfalfa aphids and the extreme susceptibility of *cv.* Hunter River. We released *cv.* Nova in May 1979 as the first aphid-resistant lucerne bred in Australia. A multi-purpose, multiple pest-resistant lucerne, *cv.* Aurora, was released in 1986. Our aim remains to breed and evaluate adapted lucernes resistant to the major factors limiting the production and persistence of lucerne in Australia.

1. Completed Program

A new lucerne, Y408 or *cv.* Aquarius, has been registered as a persistent, non-dormant lucerne for haymaking and/or grazing in irrigated or high rainfall environments. Phytophthora root rot is the major limitation to lucerne in this environment (1). Y408 is the first lucerne in Australia to be classed as highly resistant to this disease (Figure 1).

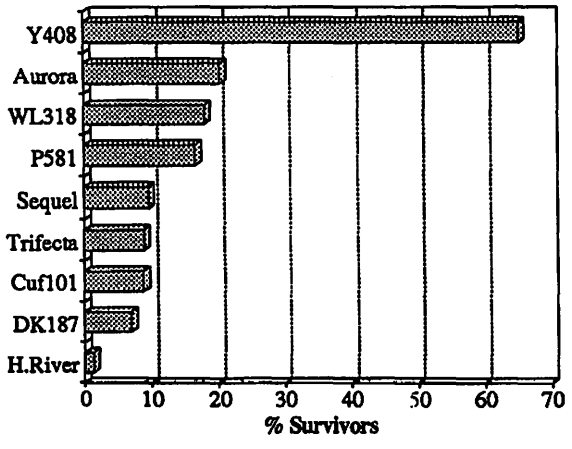

Figure 1. Resistance of seedlings of Y408 and several other lucernes varieties to death from Phytophthora root rot in the greenhouse.

Y408 is also highly resistant to damage from spotted alfalfa aphids, resistant to stem nematodes, moderately resistant to blue-green aphids, and has low resistance to colletotrichum crown rot (2). This combination of resistances allowed Y408 to persist and maintain production better than other non-dormant lucernes in irrigated and high rainfall sites in NSW. For example, Y408 averaged 110 percent of the site mean yield after two years, and 155 percent of the site mean persistence after fours years in five trials. In contrast, CUF-101 averaged 100 percent for yield and 48 percent for persistence.

2. Current Programs

An estimated 92 percent of the 1.5 million ha of lucerne in NSW is grown under rainfed conditions and usually as pastures for sheep. There is still no cultivar as persistent as the aphid-susceptible cultivar, Hunter River, in this environment. We have successfully bred several lines ("Y" numbers) which are superior to *cv.* Hunter River and other varieties in their resistance to pests and diseases and also in their persistence and productivity in dryland field trials (Figure 2). The best of these lines will be released as a new cultivar following further evaluation in the greenhouse and in field trials throughout NSW.

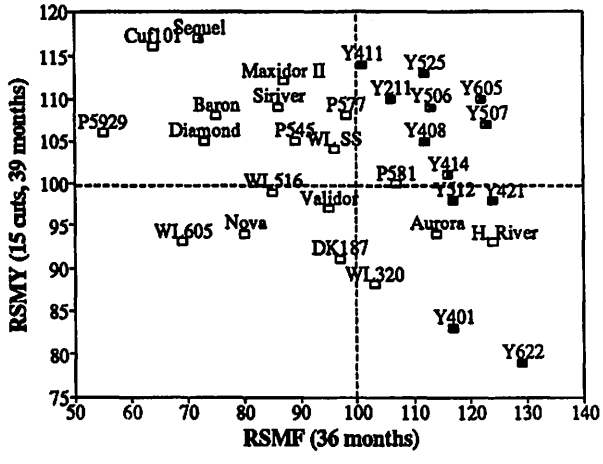

Figure 2. Total herbage yield over 39 months relative to the site mean (RSMY) and plant frequency after 36 months relative to the site mean (RSMF) for lucerne in a dryland trial at Tamworth, NSW.

Current studies also aim to identify the morphological and physiological traits most associated with the persistence of lucerne in dryland pastures. This will improve the success and efficiency of breeding ideal types of lucerne for this environment and thereby increase the benefits of lucerne to Australian farmers.

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A Brief Report on Alfalfa Land Races and New Cultivars Evaluation and Assortment in PRC

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There are 47 *Medicago* species available in the PRC, but the species most planted for animal feed is *Medicago sativa* subsp. *sativa*. *Medicago sativa* subsp. *falcata*, *M. sativa* subsp. *varia*, *M. lupulina* L., *M. polymorpha* L., and *M. ruthenica* are also used to a minor extent.

The three primary sources of *Medicago sativa* grown in the PRC are land races, new cultivars developed by breeders and ratified as national cultivars, and introduced cultivars and germplasm. There are numerous alfalfa land races in the PRC and over 60% of the alfalfa acreage is planted with these strains, however only 15 have been licensed for release to the farmer. The land races are classified as ecotypes in accordance with their local origin and growth characteristics. The Northeast the PRC Plains Ecotype is very winter hardy and produces excellent yields. The Fen-Wei River Valley Ecotype of the North and Northwest Plains is adapted to warm humid temperate areas. This ecotype produces tillers early in the spring and has small leaves. It demonstrates little cold or drought tolerance. The Yangtze-Huai Valley Plains Ecotype is very early maturing, possesses an erect growth habit, is very leafy, and quite tolerant of heat and high humidity. The Loess Plains and Vast Areas along the Great Wall Ecotype is well adapted to temperate, semi-arid climates and is the primary ecotype planted in the PRC. The Northern Xinjiang Cold Areas Ecotype is adapted to the Northern PRC and possesses medium leaf-size, matures mid to late in the growing season, has excellent winter hardiness, and is tolerant of frequent cutting. The Southern Xinjiang Oases Ecotype is adapted to the irrigated regions of southern Xingjiang and similar arid areas. It matures late in the season, has an erect growth habit, large oval leaves, variegated flowers, and yields extremely well.

There are eight new cultivars with improved winter hardiness and productivity that have been registered in the PRC: Gongnong 1 and 2 (Jilin Provincial Academy of Agricultural Science), Caoyuan 1 and 2 (Neimenggu Regional College of Agriculture), Xinmu (Xinjiang August 1st. College of Agriculture), Tumu 1 and 2 (Neimenggu Tumuji Ranch), and Gannong 1 (Gansu University of Agriculture). Improved winter hardiness may have resulted from the introgression of *Medicago sativa* ssp. *falcata* germplasm during the breeding process. With the exception of Caoyuan 2, the cultivars will likely be grown in a limited area.

The PRC had introduced approximately 300 cultivars from 26 countries. Approximately 55% of the accessions are from the US and 23% from European countries. Considerable alfalfa germplasm has been introduced from Australia including accessions of *Medicago lateralis*, *M. lupulina*, *M. polymorpha*, *M. rugose*, *M. sativa*, *M. scutellata*, *M. ternata*, *M. truncatula*, and *Chamaecytisus prolifer* (tree lucern). To this date, only 'Rambler' alfalfa has been licensed for use in the PRC.

We plan to release a book entitled *Registered Catalogue of PRC Forage Crop Plants and Cultivars* which will be published in chinese and english. The english version will be available in 1992.

Selection and Breeding of cv. Gannong No. 1 Hybrid Alfalfa (Medicago sativa x M. falcata)

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We have carried out selection and breeding work at Lanzhou on cold-tolerance and winter hardening ability in alfalfa purely for the fact that we desired for a long time to solve our lack in local legumes a serious inadequacy of protein-rich forages for animals. Through crossing between common alfalfa and yellow-flowered alfalfa by means of open-pollination and introductions of various hybrid cultivars and germplasm materials from outside Gansu Province and abroad. We have developed a new cultivar by the name of Gannong (meaning Gansu Agriculture in Chinese pronunciation) Number 1 (the original accession number is Gonnong Hybrid Alfalfa No. 18).

Materials and Methods: There are two breeding sites under the direction of Gansu Agriculture University: 1) Tianzhu Rangeland Station is situated in Tianzhu County along the so-called Corridor to the west of Yellow River Valley in Northwest Gansu. The altitude is 2990 m. above sea level with annual precipitation of 440 mm and annual mean temperature of -0.1°C. No absolute days free from frost were recorded and the lowest temperature was -29°C without and deep snow cover on windy days of both winter and spring. The soil is of alpine meadow type with a pH of 7.3. This station is a sieving base for cold-resistance breeding in alfalfa. 2) Wuwei Forage Station (Wuwei Forage Station) is situated at the eastern end of the forementioned Corridor of Hexi (in Chinese, it means river valley to the west of Yellow River). Bordering the Tengri Desert, the altitude is somewhat lower, 1660 m above sea level, with an annual precipitation of 150 mm and the same annual average temperature as Tianzhu Rangeland Station. The days free from frost were 160. The soil is a certain type of ortho-sierozem with pH = 8.5. Plant disease-resistance evaluation and seed production work was undertaken here.

In 1966 the plots were seeded at Tianzhu Rangeland Station. The initial selection of introductions and accessions took place between 1974 and 1975 those surviving this initial sieving were again screen between 1979 and 1981. Crossing and evaluation took place between 1982 and 1985 for determination of cold tolerance in the offspring. Individual plants were selected. In 1984 selected plants were clonally propagated by cuttings and the plants were divided between Tianzhu Rangeland Station and Wuwei Forage Station. Between 1985 and 1987 the clones were mixed uniformly by equal parts in plots and evaluated for further strain selection by eliminating diseased and week clones. Intercrossing was performed and the progeny of the 82 clones were mixed to constitute the cultivar Gannong No. 18 hybrid. In 1988, the cultivar was tested against other alfalfa varieties. Regional testing was conducted in 1989 and Production testing was conducted in 1990. At Tianzhu Rangeland Station in 1986 through 1987 mixed planting of progeny seed were further evaluated and cuttings were made of selected plants. In August of 1987 the selected cuttings were planted at Huangyangzhen Campus of Gansu Agriculture University. This field was interpollinated and the progeny were used in 1988 through 1990, at Wuwei Forage Station for display and extension took place.

At the start of 1989, the annual seed yield of Gonnong No. 1 was somewhat over 1000 kg/ha, then used for extension for the first time in such prefectures as Ganzhi County in Sichuan, Gannan, Sunan County and WuWei Municipality of Gansu as well as even Hainan Prefecture of Qinghai and so on. Jiamusi Municipality and Heilongjiang Province os the farthest place for extension. At the same time when breeding work is in commencement, we have studied some certain culturing technology in order to plant this cultivar for extension in cold areas of Gansu Province. The seed was available for use in those lower altitudes of 2700 m above sea level where the annual mean temperature ranges to only subzero in the neighboring regions of the Qinghai-Tibet Plateau and Loess Plateau respectively.

We wish to thank Professor Wu Renrun, LIAS, CAAS for his help in guiding and English translation work and his recommendation to NAAIC for poster session.

Performance of 33 U.S. Alfalfa Cultivars in China

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Alfalfa is one of the most important legume foyages in China. It is estimated that the sowing area is 1.0-1.2 million hectare in China. A lot of local cultivars have being used for a long time. Plant introduction has potential to success between China and United States because two countries' latitude is similar in the globe. The purpose of this trial is to observe some U. S. alfalfa cultivars adaptation in China.

U.S. alfalfa cultivars included: Dawson, Deseret, Cody, Iroquois, Lahontan, NC -83-1, Nephi, Ramesy, Rhizoma, Spreador-2, Thor, Unita, Anstar, Maxim, Hi-phy, Armor, Epic, C/W938, C/W334, C/W327, C/W118, Commandor, Durmmor, GT13R, GT49R, Victoria, Vernal, Pioneer555, 526, 581, 545, 532, and 544, The control cultivar is Gong Nong No. 1 which is one of the best alfalfa cultivars in China. The trial was conducted in the experiment farm, in the Institute of Animal Science, Beijing, in Auguest, 1986. The fertility of the soil is medium, and pH is neutrial. Four replications were arranged, with plot $2*2.5m^2$. Those cultivars were evaluated for the dry mater (DM) yield, seed yield, plant hight, regrowth ability, fall growth habit, summer hot tolerance, winter injury, pest and diseases resistance.

Through three years observation, majority of U.S. cultivars grew well in Beijing. All cultivars except GT13R, GT49R and 581 had no winter injury, All cultivars but one, Deseret survived in the summer. DM yields were quit high, ranged 12-15t/ha in general field mangement. There was no significant difference ($p>0.05$) in DM yields comparing 32 U.S. cultivars with Gong Nong No. 1. Distribuation of forage yield in growing season was not balance. According to the data of 35 cultivars, the DM yield in the first cut was highest, 43.3% of an year forage yield, but dropped to 26.6%, 16.1%, and 14.0% for the 2nd, 3rd, and 4th cut, respectivelly. It showed that the cultivars had different seasonal growth models. The aphis occured in the spring each year. No serious diseases had bappenned. Seed yields were quit poor, only 30-240kg/ha.

Preliminary conclusion: majority of U.S. cultivars performed well in Beijing. It proved that U.S. alfalfa cultivars are possible to be used in China. Comprehensively evaluation the results, some outstanding cultivars were screened out: such as Anstar, Maxim, Lahontan, C/W938, 581, Thor and Victoria. Demonstration trial should be done for the next step.

Acknowledgement: Dr Harold youngberg supported this trial.

Evaluation of genetic diversity among M.ruthenica, M.lupulina, and M.sativa germplasm resources using isoenzyme analyses

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Medicago ruthenica has a wide distribution in the north of China. It is an important wild legume germplasm resource with excellent tolerance to drought, coldness,heat and poor soil. Both of its nutritive value and palatability for animals are very good, but its aftermathing quality is not to be compared with alfalfa's. Because M.ruthenica (2n = 16) has a close blood relationship to alfalfa (2n = 32), it will be a potential germplasm resource for alfalfa's improvement in the future, its certain excellent characteristics are arising alfalfa breedists's interset.

Isoenzyme analysis is a tool often used in evaluation of genetic diversity. Ten M.ruthenicaes (Which include almost all of M.ruthenica germplams in China except Xinjiang's) were collected and seeded in our test plots. Meantime four M.sativaes and one M. lupulina wers seeded in these plots in order to survey and compare their morphological differences. Polyacrylamide gel electrophoresis method was applied in isoenzyme analysis. Analysed isoenzymes included peroxidase, esteraes, catalase and amylase. samples were extracted from stem apex of plant. The result showed that:(1)Peroxidase isoenzyme analysis indicated the clearest isoenzyme spectrum (fig.1); the secnd clearest spectrum was esterase isoenzyme(Fig.2); isoenzyme spectrums of catalase and amylase were so obecure that it was difficult to identify by photograph. (2) In these three species, isoenzyme spectrums of four enzymes signified three main isoenzyme models, this means that genetic diversities of these three species were obviously demonstrated by isoenzyme analyses. (3) In same species, although their isoenzyme spectrums were roughly unanimous, there were some obvious differences, fox example, the spectrums of Zhenmeng M.ruthenica was different from other M.ruthenicaes'.Linked their morphologic characters, they are different ecotypes which came from different regions, so thers were genetic diversities in same species. Ecotypes of these M.ruthenicaes can be divided into following four kinds: Warm-season ecotype, xeromorphy, cold-season ecotype and high altitude ecotype.

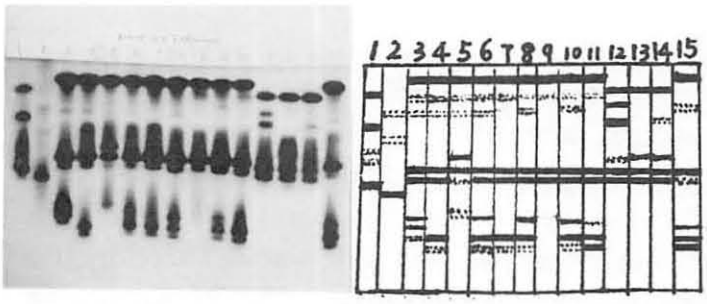

Fig.1.isoenzyme spectrum of peroxidase

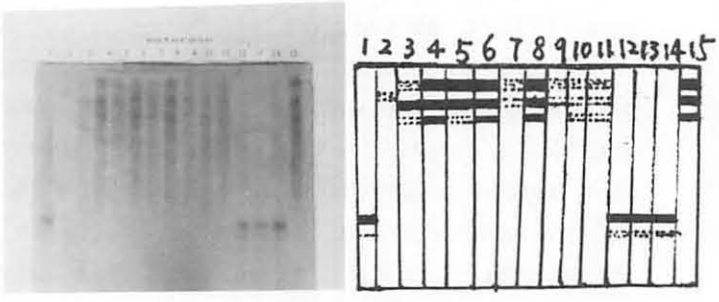

Fig.2.Isoenzyme spectrum of esterase

Denotation: MS-Medicago sativa, ML-Medicago Lupulina MR-Medicago ruthenica
1-Gongnong No.1 MS 2-ML 3-Qinshui MR 4-Shangan MR 5-Anda MR
6-Dumeng MR 7-Wumeng MR 8-Yinshan MR 9-Zhenmeng MR 10-Yanchi MR
11-Beijing MR 12-USA No.2 MS 13-Hetian MS 14-USA No.1 MS 15-Qinghai MR

Comparison of alfalfa seed germination rate in different salinities of liquor and soil

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Germinative rate of seed under saline stress is often applied to appraise its salt endurance and to use in selection of salt tolerance. Germinative experiments of alfalfa seed under different salinities of liquor and soil were conducted, we compared the differences of germinative rate under these two conditions.

Test condition:No.1 was to formulate liquors with different gradients of NaCl(sodium chloride), the concentrations of NaCl ranged from 0.0, 0.2 ··· to 2.0%, the total was twenty concentrations. No.2 was to formulate air-dry soil with different gradients of NaCl, the concentrations of NaCl ranged from 0.0, 0.2 ··· to 0.6%, the total was six concentrations, soil water content were made up into 40%, 30%, 20% respectively. Effects of salinities and water contents to seeds' germination were surveied and compared.

The rseult showed that: alfalfa's germinative rate decreased with increase of salinity, but the decreasing extent was not uniform, under low salinities, the decreasing extent of germinative rate was minor;conversely,the decreasing extent was more. Uunder stress of 0.2–0.7% salinities, their germinative rates were over 80%; under 0.8–1.0% salinities, the rates were over 60%; under 1.6% salinity, the rate was 6.8%; concentration of 1.7% NaCl inhibited absolutely seeds' germination, this is a critical concentration. It was more obvious that the speed of germination was slowed down with increase of salinities: under the condition of non-salinity, most of seeds germinated after three days(20℃), their germinative rates exceeded 80%;under 0.2% of salinity,the rate decreased only 0.1%, but their speeds slowed down, the rate exceeded 80% after four days; under 0.7% of salinity,although its rate reached 84.6%,germinative rate reached 11.7% after three days; over 80% after fourteen days, compared to the contrast, it delayed thirteen days. Thus it can be seen that under salinity, the inhibitory effect of salinity is more obvious to germinative speed than to germinative rate.

Under non-salinity soils, in 30% of water content, germinative rates of alfalfa seed was highest, the second was in 20%, the third was in 40%, their germinative rates were 100%,98.5% and 93.2% respectirly.Under stress of salinity, besides saline effect , water content is an important factor . Under 0.3% of salinity, in 40% of water content, germinative rate was 77.2%; in 30% of water content, the rate was 15.1%, in 20% of water content, the rate was only 2.3%. This showed that decrease of germinative rate was affected by both salinity and water. Therefore it is suitable to use over 1.0% of salinity in liquors and to use lower 0.3% of salinity in middle-water-content soils in appreciation of alfalfa's salt tolerance.

The Local Cultivars And Ecotypes of Chinese Alfalfa

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The alfalfa cultivation in China has the history of more than two thousand years. It has now distributed into more than 100 counties of 14 provinces in the north, ranging west from Xinjiang Autonomous Region to east to Helongjiang province, which has the distance of more than 4000 km. But its main growing areas are in the northwest and the north, less in the northeast and other parts of China. From the current statistics, the total alfalfa cultivation area in the whole country is 135.79 ha, which 88.9% of it is in the north west and the north, the other 11.1% is in the northeast and other parts. The 14 alfalfa cultivation provinces have diverse climate conditions, and through the natural and artificial selection of the long term, it gradually formed many cultivars which are adapted to the local conditions and have their unique characteristics. According to the ecological factors, the local chinese alfalfa cultivars could be divided into seven types, which could be described as the following.

1. The Oasis Ecotype in Southern Xinjiang Area

Distributed in the southern irrigation area of Xinjiang. This type has the characteristics of erect, large leaves, thick stem, light purple flowers, late maturing, high production, fast growing, good regrowth. It's suitable to grow in the irrigation area of Northern China, Such as Hexi Corridor of Gansu province, the plains in Ningxia and Hebei provinces. The main local cultivar is the Xiniang Large Leaf alfalfa.

2. The Loess Plateu Ecotype

Distributed in the middle and eastern part of Gansu province. This type has small dark green leaves, deep purple or purple flowers, the plant is semi-erect in the seasons of spring and late autumn, it is resistant to coldness and drought, has long stand persistence, relative higher production in the arid conditions. This type is suitable to grow in the Loess Plateu and the areas south of the Great Wall. The main local cultivars is Tiansui alflfa.

3. The Fen River and Wei River Vally Ecotype

Distributed in the middle of Shaanxi province and the south of Shanxi province. This type turns green earlily after winter, grows fast, matures earlily, flowers several times, has high production, but its cold and drought resistence is not very strong. It's suited to grow in the southern Loess Plateu area. The main local cultivars are Guanzhong alfala and Jinnan alfalfa.

4. The Nothern China Plain Ecotype

Mainly grows in the middle and south of Hebei province. This type is erect, has purple or light purple flowers, medium cold resisrence, grows fast, regrows well, has high forage production. It's suited to grow in the southern Great Wall area. The main local cuitivars are Baoding alfalfa, and Changzhou alfalfa.

5. The Mongolian Plateu Ecotype

Distributed in the southern Inner Mongolia Autonomous Region and northern Shaanxi province. This type has small but dark green leaves, purple or dark purple flowers, strong resistance to winterness and drought, the plant is semi-erect in the spring and the late autumn, its regrowing ability is weak. It's suited to grow in the cold and cool area. The main local cultivars are Shanbei alfalfa and Yuxian alfalfa.

6. The Northern Jiangsu Plain Ecotype

Mainly distributed in the northern Jiangsu province. It has the characteristcs of erect, leafy, early maturing, fast growing, strong heat and wet resistance. It's suitable to grow in the Yangzi & Huaihe River area. The typical local cultivar is Huaiying alfalfa.

7. The Song-nen Plain Ecotype

Distributed in the south of Heirongjiang province. This type is erect, has light purple, purple and deep purple flowers. it has strong resistance to winterness, medium production. It's suited to grow in the north eastern China. The main local cultivar is Zhaodong alfalfa.

DIFFERENTIAL EXPRESSION OF AN INSECT PROTEASE INHIBITOR GENE IN TRANSGENIC ALFALFA: EFFECT OF PROMOTER AND TYPE OF TISSUE

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The co-evolution of plants and their insect predators has produced a variety of novel plant defensive mechanisms designed to inhibit or reduce attack. Defensive strategies may include morphophysiological adaptations as well as the production of defensive chemicals which may act to both shield the plant or render its tissue unpalatable (2).

One biochemical defensive strategy employed by many genera of plants against insect attack is the production of protease inhibitors. These compounds are normally produced in the plants foliar regions in response to wounding. Protease inhibitors achieve their effect by disrupting the normal function of digestive enzymes in the insect gut, reducing the nutritional quality and digestibility of the tissue (1).

One potential approach to controlling predatory insects is to develop transgenic plants that capitalize on natural defensive strategies already utilized by plants to defend themselves against predation. This report describes our research on transferring protease inhibitor genes from an insect, the tobacco hornworm (*Manduca sexta*) into alfalfa.

Using *Agrobacterium* mediated transformation, we have transformed alfalfa with 3 insect protease inhibitor genes from *Manduca*. In this report we describe the effect of promoter construct and tissue type on one of these genes. Four different promoter constructs and leaf and root tissue were compared using Western analysis for expression level of the protease inhibitor gene. Comparisons between duplicated CaMV 35S promoter with a CaMV 19S terminator sequence and a phoshoenolpyruvate (PEP) carboxylase promoter with a CaMV 19S terminator are principally discussed here.

In the leaf tissue tested to date, the duplicated CaMV promoter has shown superior expression of the protease inhibitor gene relative to the PEP carboxylase promoter. Gene expression in clones with the PEP carboxylase promoter has been observed although signal strength was always less than that of clones carrying the duplicated CaMV promoter.

Comparisons of different tissue have confirmed expression of the protease inhibitor gene in actively growing root tissue as well although their is significant variation between various plant tissues and developmental stage. Generally, those clones which had high levels of expression in leaf tissue were also strong expressors in root tissue. Expression of the protein appears to be higher in leaf tissue than in root tissue.

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Would the alfalfa transgenic plants with human beta-interferon gene be resistant to viral diseases?

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Many approaches are known to construction of transgenic plants with tolerance to viral infection. Noteworthy are virus suppression by satellite and antisence RNA transcripts or viral coat protein expression in transgenic plants. Here we propose the new strategy to confer resistance to viral infection consisting in the expression of human beta-interferon gene in alfalfa transgenic plants. This approach is based on the effect found by Sela and others, who showed that human leucocyte interferon suppress Tobacco Mosaic Virus development in tobacco leaf disks (2).

The experimental results of alfalfa transformation by agroinfection and by direct transfer of interferon-containing constructions with the help of "growing pollen tubes" technique are presented. The following initial plant material was used for transformation: clone P868 (Mezentcev, 1981) with high level of embryogenic potential and the promising variety of siberian selection Sibirskaya-8. The co-cultivation of leaf disks with Agrobacterium tumefacience was used as well as the application of DNA solution onto stigma, taking into consideration the time need for growing pollen tubes to penetrate into the eight-nuclear embryo sac.

The most part of our experimental alfalfa plants were kanamycin resistant and NPTII-positive. Southern blot hybridization demonstrated the integration of the transferred construction into the plant genome. The preliminary tests showed that the sensitivity of NPTII-positive alfalfa transgenic progeny to Alfalfa Mosaic Virus (AMV) was changed. In the nearest future our investigations with AMV and other species of viruses carrying out together with Dr. A.Atanassov (Institute of Gene Engineering , Bulgaria) will help us to answer the question: would alfalfa transgenic plants with human beta-interferon gene be resistant to viral infection?

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The Effects of Rhizobium Inoculation and N Fertilization on Annual Medics

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Annual medics (Medicago spp.) are native to the Mediterranean region. They were introduced into Australia in the early 1900's where they have become important for ley farming, grazing and green manuring. Some annual medics have been evaluated for forage yield in dry and irrigated land in the Western USA. However, information on their growth, productivity and N fixation in the North Central USA is lacking. Our objectives were to evaluate the effects of Rhizobium inoculation and N fertilization on forage yield and nodule traits of annual medics.

A field experiment was conducted in 1991 on a Hubbard loamy sand at Becker, MN. The experimental design was a randomized complete block with four N fertilizer/inoculation treatments and eight entries in a split-plot arrangement. The main plot treatments were: inoculation, no N; inoculation, with N; no inoculation, with N; and no inoculation, no N. For N treatments, a total of 180 Kg/ha was applied in split applications at planting and one month after planting; for inoculation treatments, the seed was inoculated prior to planting and the soil was also inoculated right after planting. The experiment was established in spring on 9 May and in the summer on 1 August. The subplot treatments were eight entries including Nitro alfalfa (Medicago sativa), George black medic (Medicago lupulina L.), and six Australian annual medics belonging to five species [(M. littoralis (Harbinger), M. polymorpha (Santiago) , M. rugosa (Sapo), M. scutellata (Sava), and M. truncatula (Sephi and Borung)]. Sampling occurred on 2 July for the spring seeding and on 9 September for the summer seeding. The plants in a 0.4 m2 area were dug, washed, and roots and herbage were separated and their dry weights were recorded. The percentage of plants with nodules was calculated, and nodule mass was scored on a 0-5 scale.

For George Black medic, Nitro Alfalfa, Borung Barrel medic, Sephi Barrel medic, Sava Snail medic, and Harbinger Strand medic, over 80 percent of the plants were inoculated by the native Rhizobium, while about 50 percent of the Santiago Burr medic plants and only 15 percent of the Sapo Gama medic plants were inoculated. Inoculation increased the nodule mass of Sephi, Santiago, and Sapo compared to no inoculation, but did not increase the nodule mass of the other legumes. Inoculation increased the herbage yield of Santiago, but did not affect the herbage yields of the other legumes. As with most commonly grown legumes, N fertilizer inhibited the nodulation of medics and increased the herbage yield of Nitro, Borung, Sephi, and Sapo. Herbage yields of N-fertilized medics were frequently greater than those of inoculated medics which suggests that the effectiveness of some Rhizobium-medic associations could be improved. Root weight was not affected by N fertilizer or inoculation.

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Some Aspects of Nitrogen Fixation Improvement in the North Caucasus.

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The effectiveness of alfalfa breeding for increased symbiotic activity is conditioned significantly by genetic peculiarities of Rhizobium meliloti varieties and strains, which are applied as an initial material. The preliminary stage of breeding is to reveal the varieties with a wide range of variability to this character and well adapted to the regions of their cultivation.

The objective of our investigation was to study the varietal specificity of alfalfa, inoculated with R. meliloti.

The experiment was carried out with alfalfa clones and varieties of the Krasnodar Research Institute of Agriculture, cultivated int he Krasnodar region. The alfalfa varieties which were tested were: Sparta, Bagira and Maugli. Alfalfa seeds and rooted cuttings were inoculated with rhizotorfin (bacterial preparation ont he turf basis). The Rhizobium meliloti strains which were tested were obtained from the Research Institute of Agricultural Microbiology (Pushkin) they were 425a, CXM I-239, CXM-3, CXM I-214, CXM I-223, CXM I-251, CXM-2, and CXM 3-15. Records were made at the early flowering stage. The value of nitrogen fixation during vegetative tests was calculated by $N=N_I-N_N$, where N_I= total nitrogen in inoculated plants, and N_N= total nitrogen of the control plant which was not inoculated (Trepachev, 1981). The data obtained were calculated by the method of two-factors dispersive analysis (Dospekhov, 1979). The results showed that all of the examined cultivars are characterized by marked strain specificity on interactions in test variant. The protein yield of the inoculated varieties was superior to the control by 4.5 to 38.0% The most productive results were obtained from the interaction of Sparta and Maugli with strain CXM-3. The protein content were increased compared to the control by 36.3-38.0%. Analysis indicated that interaction between Bagira and strain CXM-I-214 was the most effective, where protein gain was 22.0%.

A group of superior active strains of nodule bacteria was revealed as the result of this two-year study. Sixty alfalfa genotypes were chosen for further studies. Firstly, the selection was made for their valuable economic characters. The plants were cloned and inoculated with CXM-I-214 strain. Further selections of clones were conducted for the effectiveness of the root system infection by the bacteria. Only 8% of the total genotypes were chosen. Clone 8549 was the most responsive to inoculation, therefore its protein yield was rather high (120%). Clones 86/14 and 8617 were also responsive to inoculation (protein gains 42.0 and 26.4%, respectively). The number of nodules and their color correlated positive with symbiosis effectiveness. The higher total productivity of clones was obtained when the nodule number increased.

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Carbon Isotope Discrimination and Water-Use Efficiency in Alfalfa Accessions

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Extensive alfalfa (Medicago sativa) production occurs in both rainfed and irrigated cultural systems. Both systems could potentially benefit from improvements in water-use efficiency (WUE), the amount of dry matter production per unit water transpired. The importance of WUE to the yield process in an alfalfa crop can be shown by the following equation,

$$\text{Yield } (gDM/m^2) = \text{WUE } (gDM/gH_2O) \times \text{Transpiration } (gH_2O/m^2),$$

where DM is dry matter. The transpiration component is related to such factors as leaf area development, water availability, diseases, insects, and soil fertility. Most yield improvements have come from the transpiration factor above; little or no improvements in WUE have been documented. Measurements of whole plant WUE requires laboriously accounting for plant water use; a process not readily applicable to genetic selection and cultivar development.

The ratio of stable C isotopes ^{13}C to ^{12}C in plant tissue differs from that in the atmosphere because ^{12}C is assimilated preferentially in the photosynthetic process compared to ^{13}C. This is termed C isotope discrimination (Δ). In recent years Δ has been shown in several C_3 crop species to be inversely related to plant WUE. The objectives of this study were to 1) determine if reproducible differences in Δ could be found in Medicago sativa germplasm of diverse origin and 2) determine if differences in Δ are related to direct measurements of plant WUE.

Eighteen Medicago sativa accessions were established in 1990 in irrigated and dryland environments at Central Ferry, WA. Significant differences among accessions were found for Δ. The environment x accession interaction was not significant indicating that Δ differences were generally consistent across environments. Values of Δ ranged from 18.93 o/oo for PI 434600, originating from Argentina, to 20.27 o/oo for PI 420400 originating from Spain. Accessions selected in 1990 for high, middle, and low Δ were sampled again in 1991. Differences in Δ were not observed in the irrigated environment but were observed in the dryland environment and were consistent with 1990 results.

Accessions with high, middle, and low Δ values from 1990 field tests were grown in pot experiments and WUE was directly measured. The high, middle, and low Δ accessions had low, middle and high shoot WUE; Δ and WUE were inversely correlated. These results show that reproducible variation in Δ does exist in alfalfa germplasm that is correlated with direct measurements of WUE. Given the range of Δ values observed and the relationship of Δ to measured WUE, an increase of 15 to 20% in WUE is appears possible in alfalfa. More work is needed, however, to determine if the relationship between Δ and WUE established on individual, isolated plants will hold in dense crop stands. Under these conditions factors associated with crop microclimate could reduce the impact of Δ on crop WUE.

Comparison of Water Applied to Evapotranspiration Estimated by Neutron Probe in a Line Source Experiment

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Field testing of alfalfa populations in a breeding program is essential to the improvement of water use efficiency in the crop. A line-source sprinkler system allows for the evaluation of plant populations under a continuous moisture gradient (1). Crop evapotranspiration (ET) may be estimated by soil samples (gravimetrically), lysimeters, the water balance equation, or neutron probe. Assuming no runoff, evaporation, or drainage, ET was estimated by measuring the water applied (WA). ET also can be calculated by measuring the soil moisture content with a neutron probe. The objective of this study is to determine whether water applied is a viable technique to estimate ET in a line source experiment.

A line source sprinkler irrigation system was used to evaluate water-use efficiency of alfalfa under three moisture levels in the field. From 17 April to 25 September 1989, ET was estimated using two techniques. The water balance equation assumes that:

$$ET = I + R - D +- SM$$

ET is calculated as the sum of irrigation (I) and rainfall (R), minus drainage (D), plus or minus the change in soil moisture (SM). To measure drainage and soil moisture changes, non-weighing lysimeters must be installed for every entry in the test. If water is applied such that drainage does not occur, and that change in soil moisture is negligible from the beginning to the end of the growing season, ET is equal to WA. With a neutron probe, soil moisture can be measured indirectly by the movement of neutrons through the soil. ET determined by the neutron probe is accurate, however, is expensive and labor intensive.

Access tubes were installed to a six-foot depth. Access tubes were read after and before every irrigation to determine ET. By reading each tube after an irrigation, field capacity can be determined. Reading each tube before the next irrigation and subtracting this value from the value obtained at field capacity, measures the ET for that period.

The results show that water applied was negatively correlated to ET as estimated by neutron probe. The assumption of no run-off, drainage, and that minimal changes in soil moisture occurred from beginning to end of the growing season, was not accurate.

The WA data illustrated that the sprinkler system applied a consistent gradient. Regression analysis showed that a strong linear relationship existed between WA and ET measured by probe with the three water treatments, R^2 of 0.98 and 0.87 respectively. WA had a steeper slope than ET measured by probe, indicating that water applied overestimated ET in the low irrigation treatment by 277%, and overestimated ET in the high irrigation treatment by 155%. For line source sprinkler experiments in the future, neutron probe data is essential for accurate determination of ET and water-use efficiency.

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Validation of Alfalfa fall dormancy in South Africa by using the AMMI model

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The Additive Main Effects and Multiplicative Interaction (AMMI) statistical model was used for the interpretation of genotype-environment interactions in alfalfa yields resulting from the National Lucerne Evaluation Program (NLEP) in South Africa. This program was designed to give an indication of the suitability of different alfalfa cultivars to environmental and agricultural conditions.

A series of 14 trials in both the Summer and Winter rainfall regions and covering both dryland and irrigated conditions were established as randomized blocks with sixteen cultivars in three replicates at each site. Data was collected in three consecutive years, 1986/7, 87/8 and 88/9 thus resulting in 42 (14 x 3) environments. The alfalfa cultivars were representative of dormancy groups ranging from 4 to 9 and originated from South Africa, USA and Australia.

From the pedigree of the alfalfa cultivars it was postulated that dormancy would be the overriding factor in determining area adaptation and that the stress factors, moisture, temperature and disease would be counteracted by lower dormancy. Using the ANOVA model these expected trends were not obvious but the application of the AMMI model resulted in rankings of cultivars validating their fall dormancy. More dormant cultivars were better adapted to environments with higher stress conditions while the non dormant cultivars had highest rankings under optimal production conditions. This trend was also evident when high rainfall during 1988 increased the potential of a specific dryland environment and influenced the ranking of the cultivars in favour of the non dormants.

A duplicate entry in a previous trial showed consistent pairing of the two entries in the rankings when using the AMMI model while this was again not evident when the normal ANOVA model was used.

The South African cultivar SA standard is furthermore, consistently outperformed by cultivars of, supposedly, similar dormancy and the AMMI rankings lead us to believe that the dormancy rating should be 4 to 5 rather than the accepted 7. This would also confirm the cultivars' adaptation to extreme grazing conditions.

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Extracting Alfalfa Root Exudates Using Supercritical Fluid Extraction

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Alfalfa is the most important cultivated forage crop in the world. Alfalfa provides livestock with protein, vitamins, and minerals. Alfalfa grown in the southwestern United States is often defecient in forage phosphorus (P) concentration. Gains from selection for P concentration has been slow (Miller et al., 1987). Several studies have implicated carboxylic acids as enhancing plant acquisition of soil P. An efficient, safe, and cost effective method of extracting carboxylic acids and other organic acids from soil media is needed to proceed with research on this important trait. Supercritical fluid extraction (SFE), a relatively new technique for analytical research, may have potential for extracting organic acids from complex matrices such as soil (Hawthorne, 1990). The objective of this preliminary research is to investigate SFE as an extraction method for organic acids found in alfalfa root exudates.

Silica sand and vermiculite samples were treated with 100 μg of citric, malic, oxalic, and succinic acids. Analytes were then extracted with supercritical CO_2, and analyzed using capillary gas chromatography-ion trap mass spectrometry. A fresh alfalfa root sample was also subjected to SFE using three different densities of supercritical CO_2 to determine if selective extraction was possible.

To date, extraction of carboxylic acids from silica sand and vermiculite has been unsuccessful, even when chemical modifiers were added to the sample prior to extraction. Further study showed that the acids had become bound to soil sites by carboxyl and hydroxyl groups. Analysis of alfalfa root extract showed that different compounds were extracted by changing supercritical CO_2 density. Currently, we are investigating how sample pH, temperature, and supercritical CO_2 flow rate affect the solubility of carboxylic acids. Supercritical fluid extraction should prove to be a valuable extraction methodology for physiological research in agricultural crops.

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In Vitro and In Situ Evaluation of Near Infrared Selected and Unselected Alfalfa Lines

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Near infrared reflectance spectroscopy (NIRS) is quickly becoming accepted as an accurate, rapid, and cost-effective method for determining forage quality in alfalfa. With proper calibrations, NIRS has the ability to accurately characterize crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) in alfalfa on a genotype basis. This ability to quickly and accurately characterize important forage quality parameters has led alfalfa breeders to begin using NIRS for routine screening and selection for higher CP, lower ADF, and lower NDF. However, little information is available on whether NIRS-selected alfalfa lines meet three important criteria: higher forage quality as measured by NIRS; higher forage quality as measured by standard chemical analysis; and improved animal digestibility as measured by in-situ rumen degradation. It will become important for alfalfa breeders using NIRS-based selection procedures to demonstrate that their "high quality" lines produce improved animal performance when compared to unselected materials. Our objective was to determine whether differences exist between NIRS-selected and unselected lines for NIRS, chemical, and in-situ forage quality and digestibility parameters.

A solid-seeded yield trial (3' x 20' plots, four replicates) at Evansville, WI was sampled for quality on July 30 and August 30, 1990. All plots were at the late bud (MSC=3.9) stage when harvested. Alfalfa entries included Unselected A, a commercially available semi-dormant cultivar with above average forage quality; Unselected B, a commercially available semi-dormant cultivar with average quality; and NIR Select, a cultivar developed from Unselected A following one cycle of selection for high CP, low ADF, and low NDF using NIR spectroscopy. Forage samples were dried, ground and analyzed for CP (Kjeldahl procedure), NDF, ADF, and ADL (Robertson and Van Soest, 1980). Samples were also analyzed for CP, ADF, and NDF using a Pacific Scientific Model 4250 NIRS. Three dry pregnant cows fitted with a rumen cannula were used for in-situ analysis. Dacron bags were used to incubate 2.5 g of dry matter per sample. For each treatment, a series of nine bags was incubated in the ventral region of the rumen of each cow for 2, 4, 8, 16, 24, 32, 48, 72, and 96 h. Degradation curves were then developed from bag residuals.

Significant differences were observed between NIR Select and Unselected B for CP, ADF, and NDF as measured by NIRS. Non-significant differences were observed between NIR Select and Unselected A for CP, ADF, and NDF. However, in all comparisons NIR Select demonstrated improved quality (higher CP, lower ADF and lower NDF) when compared to either unselected cultivar. Significant differences were also observed between NIR Select and both Unselected A and Unselected B for CP, NDF, and ADF, as measured by chemical analysis. In addition, NIR Select and Unselected A were significantly lower in ADL than Unselected B. In general, chemical analysis of NIR Select suggested that one cycle of NIRS-mediated selection produced a line with significantly higher forage quality (higher CP, lower ADF, lower NDF) when compared to the parental (Unselected A). NIR Select possessed significantly less indigestible dry matter at the end of in-situ incubation when compared to both Unselected A and Unselected B. Significant differences were also observed for whole plant solubility between NIR Select and Unselected B. Percent degradable dry matter was similar for both NIR Select and Unselected A; Unselected B demonstrated significantly lower degradable dry matter. Estimated rumen digestibility (soluble + degradable) in NIR Select was significantly greater than Unselected B and numerically greater than Unselected A. In general, the rumen digestibility of NIR Select and Unselected A were similar to each other and significantly greater than Unselected B. The major impact of NIRS-mediated selection on in-situ degradability in this study appeared to be a significant reduction in the indigestible pool in selected material (NIR Select) when compared to the parental line (Unselected A).

In this study, three cultivars, harvested at the same maturity, displayed significant differences for forage quality and in-situ degradation. It appears that NIRS-mediated selection for CP, ADF, and NDF can be an effective way to improve the chemical composition and rumen degradation properties of alfalfa. When measured using chemical analysis, the NIRS-selected line was significantly higher in CP and lower in fiber when compared to the parental line. In addition, the selected line possessed a significantly smaller indigestible pool at the end of rumen incubation when compared to the parental line. In summary, it appears that significant improvements in alfalfa forage quality are possible using NIRS selection techniques. However, the ultimate challenge will be to demonstrate improved animal performance (more milk, beef, etc.) per ton fed of these selected "high quality" alfalfas. In this study we present preliminary results from in-situ degradation studies suggesting that NIRS-selected lines can demonstrate improved rumen degradability characteristics.

Correlations Among Six Diseases of Alfalfa, *Medicago sativa* L.: Implications for Breeding

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Fusarium avenaceum (Fav), *F. oxysporum* (Fo), and *F. solani* (Fs) are common organisms isolated from rotted alfalfa crowns in New York (Salter, 1985). Common genetic mechanisms may condition resistance to crown rot caused by these pathogens, and perhaps even to other vascular diseases such as Fusarium wilt (FW) (caused by *F. oxysporum* Sch. ex Fr. f.sp. *medicaginis* [J.L. Weimer]), bacterial wilt (BW) (caused by *Clavibacter michiganense* subsp. *insidiosum* [McCull.]), and Verticillium wilt (VW) (caused by *V. albo-atrum* Reinke & Berth.). Our objectives were to determine if levels of resistance to six alfalfa diseases within two plant populations are correlated, and to determine if these associations may allow more efficient selection.

Two Flemish plant populations, developed from crosses 'Reselect Saranac x Vertus' and '(Flamande x Saranac AR2)An', were random mated toward equilibrium to produce NY9129 and NY9130, respectively. We suspected that these two populations had large genetic variability for the vascular diseases because of the moderate levels of resistance to the diseases studied. Half-sib seed produced on NY9129 and NY9130 was used in disease reaction evaluations involving the following plant population-pathogen combinations:

NY9129: Fav, Fo, Fs, BW, FW, and VW
NY9130: Fav, Fo, Fs, and FW

Each disease evaluation was set up in a randomized complete block design with three replicates, using 92 and 81 half-sib families per evaluation for NY9129 and NY9130, respectively. Additive genetic correlation coefficients and their corresponding standard errors were computed on a half-sib family-mean basis for each plant population-pathogen combination. Narrow-sense heritabilities and their standard errors were computed as well.

Significant additive genetic correlations (≥ 0.49) were found between the disease reactions for all three crown-rotting species of *Fusarium*. No consistent correlations were found between crown-rotting fusaria and the other pathogens. However, diseases caused by Fav, Fs, and FW were significantly correlated (.32 to .67) with VW in NY9129. On a half-sib family-mean basis, heritabilities for the diseases caused by the three *Fusarium* species associated with crown rot ranged from 0.21 to 0.53, compared to 0.90 for FW, 0.92 for BW, and 0.49 for VW. Based on additive genetic correlations and heritability estimates, there were no instances where indirect selection for disease resistance would be more effective than direct selection. Selection using just one of the *Fusarium* species tested should increase resistance to the other genetically-correlated *Fusarium* species. Because of the high associations, selection for resistance to the diseases caused by the crown-rotting fusaria probably could be accomplished efficiently by inoculating plants with a mixture of the three *Fusarium* species. Besides drift, selection for resistance to disease caused by the three crown-rotting fusaria should not reduce levels of resistance to FW, BW, and VW in alfalfa populations.

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Alfalfa Population Improvement for Resistance to *Aphanomyces euteiches*

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Aphanomyces euteiches Drech. is present in many of the soils in which alfalfa is grown. *A. euteiches* is known to cause seedling death and stunting in mature alfalfa plants. Recent investigations (1) have observed a wide range of variability in virulence of *A. euteiches* isolates on alfalfa. The reaction of alfalfa to different isolates is also varied, ranging from susceptible to highly resistant. The most economical control of Aphanomyces root rot in alfalfa is host resistance. The University of Wisconsin has developed the resistant germplasm WAPH-1 (2) (approx. 50% resistant) from a strain cross of two populations. Since the development of WAPH-1, other *A. euteiches* isolates (NC-1 and WI-98) have been found to be virulent on WAPH-1 causing a susceptible host phenotype. WAPH-1 is resistant to isolates that are in "Group-1" (ex. MF-1). An evaluation of 1189 alfalfa Plant Introductions (PI) was used to indentify resistant genotypes to Group-1 isolates. Of those PI's that were >5% resistance to Group-1, a second evaluation with "Group-2" (ex. NC-1, MS-13, ID-45) isolates was performed. Four PI's were chosen to develop populations with higher levels of resistance to Group-2 isolates (439006, 468018, 206572, and 464781)(Table 1). All four PI populations increased in resistance to Group-2 (NC-1) isolates from cycle 0 to cycle 2. A third isolate (WI-98) was also used to evaluate the four PI's. None of the PI's, except for C2 of 439006 had any level of resistance to WI-98. These results may indicate that a third group of the alfalfa strain may exist. The PI 439006 had a higher average resistance to NC-1 than the other three PI's. One plant from the PI 439006 produced selfed progeny that were 45% resistant to Group-2 isolates. This plant and other plants from the other three PI numbers with a higher frequency of resistance to Group-2 isolates were intercrossed to agronomically improved germplasm (Col-1 and WIS-3). Transfer of resistance to 33 different crosses (PI's x Col-1 or WIS-3) ranged from 0 to 20% resistance. In summary, *Aphanomyces euteiches* resistance (Group-1 and 2) is moderately to highly heritable. Therefore, phenotypic recurrent selection can increase resistance in many alfalfa populations. Transfer of resistance from unadapted germplasm (PI's) can successfully increase the frequency of resistance in adapted germplasm.

		ISOLATES							
		MF-1		NC-1		WI-98		BULK	
PI#	Cycle	DSI	% R	DSI	% R	DSI	% R	DSI	% R
439006	C0	4.53	1.1	4.11	0.0	4.27	0.0	4.47	2.3
439006	C1	3.10	34.2	3.58	8.3	4.10	0.0	3.92	4.7
439006	C2	3.06	36.8	3.00	28.2	3.89	2.1	3.77	10.2
468018	C0	4.10	6.9	4.05	0.0	4.50	0.0	4.43	0.0
468018	C1	3.45	24.6	3.69	4.9	4.06	0.0	3.47	9.3
468018	C2	2.89	44.3	3.59	7.0	3.92	0.0	3.45	11.3
206572	C0	4.37	0.0	4.08	0.0	4.37	0.0	4.56	0.0
206572	C1	3.49	20.9	3.63	1.4	4.32	0.0	3.75	1.8
206572	C2	3.67	10.1	3.72	1.0	3.92	0.0	3.93	3.7
464781	C0	4.09	4.5	4.01	0.0	4.42	0.0	4.41	0.0
464781	C1	3.39	21.4	3.64	3.0	4.11	0.0	3.89	5.6
464781	C2	3.26	27.2	3.33	12.7	4.05	0.0	4.15	5.7

C.V. = 6.78; Mean LSD = 0.25

Table 1. Mean Disease Severity Index (DSI) and Percent Resistance (% R) for three cycles (C) of selection in four Plant Introductions (PI) for resistance to three isolates of *A. euteiches*.

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Reaction in Some Alfalfa Populations to A New Isolate of Peronospora trifoliorum

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Pathogenic specialization occurs in Peronospora trifoliorum d By., the causal fungus of alfalfa downy mildew (DM) (3). Using Standard Test procedures (4) we compared the virulence of isolate I-9, isolated in 1991 from alfalfa growing near Chino, CA, with I-8 (3) collected near El Centro, CA, in 1977, and I-7 from KS. Also, we compared DM resistance among the broad-based alfalfa germplasm populations representing the nine historical diversity groups (2) (PI's in Table 1) and some cultivars and germplasms representing certain diversity groups. 'Caliverde' is 90% Chilean, 'Sonora' is 100% African, 'Mesa Sirsa' and KS189 are 100% Indian, and 'Saranac' is 87% Flemish. UC 193 was derived from broad-based 'CUF 101' (53% Chilean, 23% Indian, and 11% Turkistan) plants selected for resistance to isolates I-7 and I-8. KS208 (55% Flemish and 29% Chilean), selected for resistance to isolates I-7 and I-8, is a recommended resistant DM check (4).

Table 1. Percentage of alfalfa seedlings resistant (symptomless) to three isolates of Peronospora trifoliorum

Alfalfa population		Crop Sci. (Reg.)	Isolate I-7	I-8	I-9
M. falcata[1]	PI 560333	--	78.8	75.6	40.6
Ladak	PI 536532	30:753	14.2	7.4	5.5
M. varia	PI 536533	30:753	14.0	19.5	6.7
Chilean	PI 536534	30:753	51.1	7.0	5.9
Peruvian	PI 536535	30:753	17.8	0.0	0.0
Indian	PI 536536	30:753	21.4	3.9	4.3
Turkistan	PI 536537	30:753	12.9	11.9	4.3
Flemish	PI 536538	30:753	33.2	44.2	19.7
African	PI 536539	30:753	40.8	12.6	0.0
'Sonora'	FC 45010	4:665	58.4	11.4	0.0
'Caliverde'	FC 45020	[2]	45.9	8.2	4.9
'Mesa Sirsa'	FC 45011	8:396	29.9	1.1	0.0
KS189		26:204	80.8	37.6	12.6
'CUF 101'	FC 45030	23:398	39.0	4.8	0.0
UC 193		28:578	88.5	77.3	8.4
'Saranac'	FC 45009	6:611	15.4	31.9	12.2
KS208		29:1094	86.5	82.5	51.6
'Kanza'	FC 45008	9:847	0.0	0.7	0.0
		LSD(0.05)	8.5	5.1	4.7

[1]Medicago sativa subsp. falcata cv. WISFAL (1)
[2]Agronomy J. 50:664

Pathogenically, I-9 appears more similar to I-8 than to I-7 but more virulent than I-8 (Table 1). Resistance to I-7 was broad-based whereas only populations with M. falcata or Flemish germplasm expressed substantial levels of resistance to I-9. The DM resistance of UC 193 was largely overcome by I-9 whereas that of KS208 (55% Flemish) prevailed. The discrepancy between Saranac FC 45009 resistance to I-8 (31.9% in Table 1 vs. 52.4% (3) and an expected range of 50-60% (4)) may trace to the seed. The newly acquired Saranac FC 45009 seed used in this test was compared with remnant FC 45009 seed received in 1985 and 1989. The percentages of plants resistant to I-8 were 50.2±7.1, 56.1±3.5, and 25.6±5.6 for the 1985, 1989, and 1992 seed lots, respectively.

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Inheritance and Recurrent Selection for Resistance to Spring Black Stem and Leaf Spot in Alfalfa

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Spring black stem (SBS) is a destructive alfalfa disease in the U.S.A. Phoma medicaginis Malbr. & Roum. is the common cause of stem blackening (2). The development and use of resistant cultivars might be a practical control method. Our objectives were to study the inheritance of resistance to SBS in the cultivars 'Oneida' and 'Vertus', through both quantitative and qualitative analyses, and to determine the gain from two cycles of selection in the populations ('Pinnacle + Oneida VR') and 'Sabre'.

Three-week old alfalfa seedlings were inoculated with a suspension of 10^6 spores/ml of P. medicaginis. The inoculated plants were placed in a dark, moist chamber at about 20 ^{o}C for 72 hours. Disease severity on leaves was evaluated visually on a 1 to 5 scale (1 indicating symptomless plants), 18 days after inoculation. For the inheritance study, parents were chosen on the basis of half-sib progeny performance. Quantitative analyses were done on three sets of complete diallels. Genotypes were considered fixed effects, with each set represented by two plants appearing resistant, two moderately resistant, and two susceptible. For the qualitative analysis, resistant x resistant, resistant x susceptible, and susceptible x susceptible crosses were made. From the diallels, parent plants were selected on the basis of half-sib progeny performance to develop more resistant and more susceptible populations.

The GCA effect for disease reaction, when sets were pooled, was significant in both populations. The segregation patterns in the three types of crosses of the qualitative analysis appeared to follow a normal, unimodal distribution and did not appear characteristic of simple inheritance. Therefore, the study suggests the hypothesis of an additive model for resistance to SBS and an absence of non-additive genetic effects in the inheritance of the trait. Heritability on a parent-offspring basis was almost 0 in both cultivars; however, on a half-sib progeny basis, heritability was 0.26 and 0.39 for Oneida and Vertus, respectively. In both populations, the expected gain was almost as twice as much for half-sib progeny selection as for phenotypic selection. Two cycles of recurrent phenotypic selection in either population were not effective. One cycle of half-sib selection was enough to significantly increase the level of resistance to SBS. Thus, the average disease severity was 3.10 and 3.48 for the resistant and susceptible populations, respectively. These results support those of Haag and Hill (1), indicating half-sib progeny selection as a better method to exploit genetic variability in a breeding program.

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A Method to Screen and Evaluate Alfalfa for Seedling Resistance to Pythium spp.

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Pythium spp. are major soilborne fungi associated with seed rot and seedling damping-off of alfalfa. Hancock (2) reported variability among alfalfa cultivars in response to rootlet infection by P. irregulare, and suggested that genetic resistance or tolerance could be used to manage Pythium root diseases. A culture plate method has been used to select alfalfa germplasm for resistance to seedling damping-off caused by Rhizoctonia solani Kuehn (1). The objective of this study was to develop a laboratory method to screen and evaluate alfalfa germplasm for resistance to Pythium seed rot and seedling damping-off.

Culture plate method: a 3mm-diameter disc is removed from the periphery of a 2 day-old Pythium culture growing on corn meal agar, placed in the center of a 9cm-diameter petri plate containing 1.5 percent water agar and incubated at 24° C for 3 days. Twenty-five seeds for evaluation studies or 100 seeds for selection experiments are spaced on the inoculated agar surface with a vaccuum seed head. The plates are incubated at 18° C, 14-h daylength, for 5 days. Disease severity is rated using a 1 (healthy seedling) to 5 (rotted seed) scale. Resistance is characterized by an Average Severity Index (ASI = Numerical value of class x number of individuals in class / number of seeds expected to germinate) and Percent Resistant Plants (PRP = classes 1 + 2).

Pathogenicity, selection and evaluation experiments were conducted using this method. Fourteen isolates of Pythium spp. from MN and WI alfalfa field soils were evaluated for pathogenicity to the Beltsville International Composite-7 (BIC-7) alfalfa population, using three temperatures: 12, 18, and 24° C. Two-hundred fifty-five North American cultivars also were evaluated for seedling resistance using three isolates differing in pathogenicity and morphology.

A wide range in pathogenicity was found between isolates of Pythium spp. There was a significant interaction between isolates and temperature. Moderately low virulent isolates induced greater disease severity at lower temperatures than at high temperatures. PRP in the BIC-7 population varied ($P < 0.05$) for different isolates from 0 percent to 50 percent. Alfalfa cultivars varied for PRP from 25 percent to 0 percent and for ASI from 3.25 to 5.00 for different isolates. The results of these experiments suggest that this method may be useful for screening and evaluating alfalfa for seedling resistance to most Pythium spp.

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Random Amplified DNA Fragment Length Polymorphisms Among Stemphylium Pathogens of Alfalfa

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Historically, Stemphylium leaf spot of alfalfa (Medicago sativa L.) was attributed to Stemphylium botryosum Wallr. and its ascosporic state, Pleospora herbarum (Per.:Fr.)Rabenh. However, Simmons (1) has concluded that S. botryosum and P. herbarum are not components of the same holomorph and has recognized three Stemphylium/Pleospora holomorphs from alfalfa:S. botryosum/P. tarda, S. herbarum/P. herbarum and S. alfalfae/P. alfalfae; and S. globuliferum and S. vesicarium. We investigated, at the molecular level, genetic diversity and phylogenetic relationships among isolates of these Stemphylium species provided by Simmons and from alfalfa from diverse geographic areas from the United States. Genomic polymorphisms were detected by the random amplified polymorphic DNA (RAPD) method (2). Data were analyzed using an unrooted phylogenetic tree and principal component analysis of 205 RAPD fragments generated from genomic DNAs of 26 Stemphylium monoconidial isolates with 11 primers.

Principal component analysis grouped the five morphology-based taxonomy species into two clusters. One cluster included S. botryosum and S. globuliferum. The other cluster included S. alfalfae, S. herbarum, and S. vesicarium. Pithomyces chartarum and P. atro-olivaceous, included in the analysis as outgroups were separated from Stemphylium and from each other. One major RAPD fragment was common to all Stemphylium species but absent from Pithomyces. Of the 12 Stemphylium isolates from alfalfa from the United States, one isolate each from New Hampshire and Pennsylvania were identified as S. botryosum and the other eight, from California, Idaho, Kansas, Utah, Washington, and Wisconsin, were S. alfalfae. Phylogenetic analysis (Fig. 1) indicated that S. botryosum, and S. globiliferum were divergent from S. alfalfae, S. herbarum, and S. vesicarium.

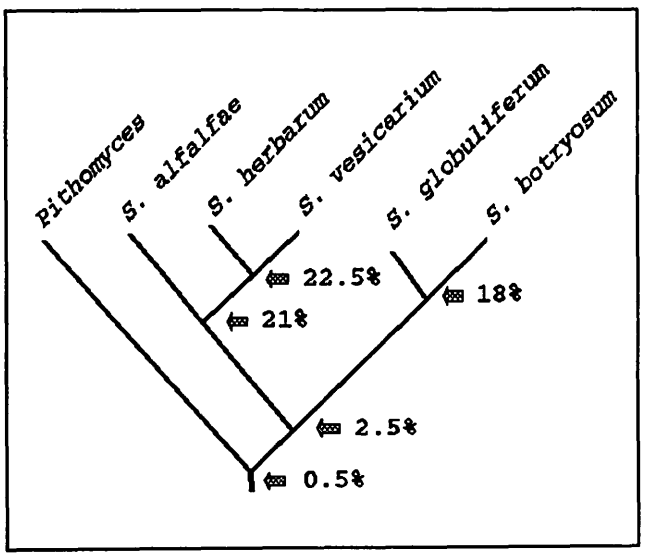

Fig. 1. Phylogenetic tree based on RAPD fragment polymorphisms of Stemphylium species from alfalfa, and Pithomyces included as an outgroup. Percentages indicate the portion of RAPD fragments in common between branches.

These results corroborate Simmons' conclusion that at least five Stemphylium species cause leaf spot of alfalfa (1). Therefore, we suggest that Stemphylium leaf spot of alfalfa is a complex host-parasite interaction involving at least five evolutionarily distinct species of Stemphylium. The RAPD method yielded markers unique to particular species, suggesting this technique may be useful as a diagnostic tool.

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Recent Outbreaks of *Rhizoctonia* Diseases of Alfalfa in Kentucky

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Separate outbreaks of web blight of foliage and stem canker on basal portions of shoots were observed in numerous alfalfa fields during warm, wet conditions in Kentucky during 1991. Isolations consistently yielded *Rhizoctonia*-like fungi. Three representative isolates each from plants exhibiting symptoms of web blight and stem canker were characterized as *R. solani* AG-1 (intraspecific group 1-B) (1) and *R. solani* AG-4, respectively. Koch's postulates were fulfilled for both diseases. Although both web blight and stem canker have been reported previously on alfalfa (2), AG types of *R. solani* have not been reported. Outbreaks of stem canker were observed in a variety of rotation sequences and tillage systems. However, the most severe outbreaks occurred where the previous crop was a grass (sod or warm-season annual grass). This was particularly evident in an alfalfa field where symptoms of stem canker were severe in the section previously cropped to a bluestem sod but minimal in the section previously cropped to tobacco. Four months later, stand counts in this field did not differ ($P>0.2$) between the section severely affected by stem canker vs. the unaffected section. However, another grower reported severe stand loss in a field where laboratory examination of plants revealed no biotic stresses other than Rhizoctonia stem canker. It is not known whether stand loss is usually associated with an outbreak of Rhizoctonia stem canker of alfalfa. However, such an association seems possible since *R. solani* AG-4 also infects taproots of alfalfa (2).

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Bidirectional Selection for Resistance to Sclerotinia Crown and Stem Rot in Alfalfa (Medicago sativa L.)

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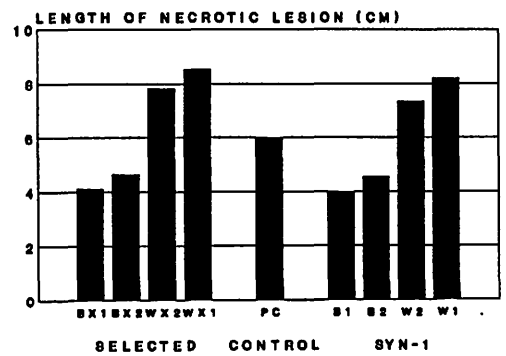
Sclerotinia crown and stem rot is a major disease in alfalfa caused by either Sclerotinia trifoliorum Eriks. or S. sclerotiorum (Lib.) de Bary. Presently, some alfalfa cultivars sustain less severe disease than others; however, resistance of economic importance is not available. Several methods of evaluation also have been developed, but the lack of two extreme resistant and susceptible populations hinders the progress of the research. This research was to determine the response to a single cycle of bidirectional selection for resistance and for susceptibility to Sclerotinia crown and stem rot and to develop alfalfa germplasm to use as resistant and susceptible checks.

Twenty-five plants in each of four source populations (Apollo, Arc, Bic7-cls5, and Delta) were previously evaluated (Aung, 1990). Based on his measurement, selections were made for resistance and susceptibility in each source population at 2 levels of selection intensity, 4% (1 plant in 25) and 8% (2 plants in 25). Selected parents in each level of selection intensity were intercrossed by hand without emasculation to produce the first generation of Synthetic Populations (Syn-1). B1 and B2 are Syn-1 populations derived from intercrossing resistant parents at 4% and 8% level of selection, and W1 and W2 are Syn-1 populations from intercrossing susceptible parents at 4% and 8% level of selection respectively. These four Syn-1 populations along with clones of each respective parents (BX1, BX2, WX1, and WX2) and a control population (PC) from remanent seeds of each source population were grown in the greenhouse. The plants in each population were evaluated by the "Stem-Tip Inoculation Method" using the Alf-2 isolate of S. trifoliorum (Pratt and Rowe 1991). The mean value of necrotic lesion in each population was measured as 80 plants consisting of an equal number of plants from each source population.

FIG.1. MEAN VALUE OF NECROTIC LESION IN SELECTED PARENTS, CONTROL, AND SYN-1 POPULATIONS



Results indicated the response to selection was significant in both directions at 4% and 8% level of selection. The mean values of Syn-1 populations were symmetric about the mean value of control population (PC) and were not different from the mean values of respective parents (Fig.1). Since previous research (Aung, 1990) indicated that resistance to S. trifoliorum was a quantitative trait controlled by many relatively minor genes, this suggested that most probably each allele controlling this trait contributes an equal effect to one another. Realized heritability in each Syn-1 population, calculated from the relationship of selection intensity, standard deviation of control population, and response (Falconer, 1989), ranged from 30% to 46%.

This selection was effective in increasing both resistance and susceptibility of progenies measured by stem-tip inoculation method and hopefully will lead to development of more effective and efficient screening method and development of alfalfa that is resistant to Sclerotinia crown and stem rot.

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Approaches to Control of Sclerotinia trifoliorum in Crimson Clover and Alfalfa by Cultural Practices and Host Plant Resistance

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Research in the USDA, ARS, Forage Research Unit at Mississippi State over the past nine years has been directed toward development of cultural control practices for disease caused by S. trifoliorum in crimson clover, and of resistant germplasm in alfalfa, crimson clover, and berseem clover.

Foliar clipping treatments, applied at select points in the disease cycle, provided significant cultural control of S. trifoliorum on crimson clover. Cutting and removal of foliage in early November, prior to apothecium formation, reduced disease severity and increased yields consistently over years. Cutting in January, after apothecium formation, gave more variable results, and cutting in February was ineffective (1).

Wide-row plantings of crimson clover developed less disease than narrow-row or broadcast plantings, but yields were not always increased because broadcast plantings are higher-yielding. Results to date suggest that use of wide-row plantings may both reduce disease losses and increase yields in comparison to broadcast, but only at relatively high levels of disease.

A stem-inoculation technique to screen for resistance to S. trifoliorum in alfalfa has been described (2). To evaluate effectiveness of this technique, 500 plants of cultivar Delta were screened for resistance by repeated inoculations of excised stems, and four plants considered relatively resistant were selected and polycrossed. The three most resistant half-sib families were evaluated for whole-plant resistance. All three families manifested significant resistance, in comparison to Delta, with controlled inoculations of foliage in a growth room. A mixed population of these three families also manifested significant resistance, in comparison to Delta, in two experiments in the field. These results indicate that the stem-inoculation technique enables selection of heritable, whole-plant resistance to S. trifoliorum in alfalfa.

To develop resistant and susceptible check lines, selections were made in three alfalfa cultivars (Arc, Apollo, and Delta) and one germplasm (BIC-7 CLS5) by use of the stem-inoculation technique (2). The Syn-1 progenies produced from selections for resistance and susceptibility were both significantly different from the original populations. Broad-sense heritability estimates were in the range of 30-46%.

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Responses of Legume Seeds and Seedlings to Exudates of Sclerotinia sclerotiorum and S. trifoliorum and comparable low pH medias

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Sclerotinia crown and stem rot caused by Sclerotinia trifoliorum and S. sclerotiorum on legumes can be an economically devastating disease in the humid South. Historically, using the disease causing organism in screening for resistance in the seedling stage has not appeared effective. One alternative has been selection for resistance to oxalic acid damage in seedlings which has not appeared to be always effective. Oxalic acid is thought to be important in this disease. The objective of this research was to assess the importance of oxalic acid in exudates of either fungus on different legume cultivars.

As potential hosts, were four cultivars each of alfalfa (Medicago sativa), crimson clover (Trifolium incarnatum), and berseem clover (T. alexandrinum). A host-pathogen interaction system (HPIS) using the Lutri Plate (Tomaso-Peterson and Krans, 1990) with a 0.2 micron filter kept fungus from infecting seeds while allowing migration of fungal exudates to the seed. Seed was exposed to exudate from a fungus or put on PDA media at pH 6.2 (the control), on PDA ammended with HCl to pH 3.2, or PDA ammended with oxalic acid to pH 3.2. The fungi reduced the pH of germination medium in the Lutri plate to 3.2. After seven days, measurements were made of percent germination and length of germinated seedlings.

In this design, HCl treatment is an effect due to lowered pH, the oxalic acid shows effect of lowered pH plus effects associated with this acid, the fungal effects show acidity effects, oxalic acid effects, and the effects of other undefined materials in the exudate. The acids did not affect percent germination, but the fungi had small significant (P=0.05) effects. Seedling length was affected by all treatments. The exudate of S. sclerotiorum reduced seedling length 60 % and the exudate of S. trifoliorum reduced it by 51%. For S. sclerotiorum the oxalic acid and HCl account for 58% and 27% of reduction in length, respectively. For S. trifoliorum, oxalic acid and HCl account for 68% and 31% of reductions, respectively. Over the 12 cultivars, the oxalic acid response correlated with S. sclerotiorum (r=.83) and S. trifoliorum (r=.71). No correlations with percent germination were significant while all were significant for seedling length (r = .56 to .84).

A quantification of the importance of reduced pH, oxalic acid, and fungal exudate showed oxalic acid was important in reduction of seedling length for alfalfa and crimson clover, not berseem. The significant differences among alfalfa cultivars to fungal exudates and oxalic acid; the correlation of oxalic acid response with either fungus, and the magnitude of effect by oxalic acid in decrease of seedling length by either fungual exudate suggest in total that screening for seedling length in presence of oxalic acid might still be useful selection criteria or technique for reducing the damage caused by fungal exudates in alfalfa.

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Incidence of Pea Enation Mosaic Virus in Alfalfa in Washington State

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Alfalfa (*Medicago sativum* L.) was first reported as a host for PEMV by McEwen and Schroeder in 1956 (2) and has been considered the perennial host following a survey by McWhorter and Cook in 1958 (3). This was recently reaffirmed in a survey by Rahman (4) but other workers have disputed the above reports (1, and W. J. Kaiser, USDA-ARS, personal communication). The objective of this survey was to confirm the occurrence of PEMV in alfalfa in areas where it had been previously reported.

Alfalfa samples were collected from fields in central and western Washington. In addition, dried samples collected and prepared by Rahman during 1987 and reported to be infected with PEMV (4) were re-examined. All samples were assayed by double antibody sandwich ELISA using antisera against PEMV prepared either by R. O. Hampton (USDA-ARS, Corvallis, OR) or G. deZoeten (University of Michigan, East Lansing, MI).

PEMV was not detected by ELISA in any of 665 alfalfa samples collected in this survey (35-80 samples from each of 14 fields); however, alfalfa mosaic virus and bean leafroll virus were successfully detected. PEMV was not detected in dried samples that had been previously reported to be infected with the virus (4). With the exception of McEwen and Schroeder (2) and Rahman (4), there has been no evidence presented by other workers demonstrating by vector transmission, serological, or cDNA/RNA probe hybridization techniques that PEMV is present in alfalfa. McWhorter and Cook (2) were unable to recover PEMV from overwintering alfalfa in Washington and Oregon by either mechanical or aphid transmission although they accepted the work of McEwen and Schroeder. Their conclusion that alfalfa was the principal perennial crop responsible for the spread of PEMV to adjacent pea fields was supported only by observation and circumstantial evidence. Hagedorn et al. attempted to transmit with aphids a California isolate of PEMV to alfalfa and white clover; however, their attempts to recover the virus from these hosts proved unsuccessful (1). PEMV was reported to be widespread in alfalfa in the Puyallup, Coupeville, and Gardena Bench areas of Washington (4), but these findings were not corroborated when the original dried or freshly collected samples were assayed using DAS-ELISA. Attempts have been made to transmit PEMV isolates into and out of alfalfa using the pea aphid (*Acyrthosiphon pisum*) but without success (R. C. Larsen, unpublished, and W. J. Kaiser, personal communication). It might be argued that the susceptible alfalfa varieties cited previously (2) are virtually no longer grown commercially and that PEMV cannot be detected in newer varieties. However, this appears unlikely considering that the virus could not be detected even in the "susceptible" (2) 11- and 21-year-old Vernal alfalfa fields examined in this study. The results of this survey combined with the current inability to transmit PEMV to alfalfa strongly suggest that alfalfa is not a host and consequently should not be considered a perennial host for this virus in Washington State or elsewhere.

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Individual and Combined Inoculations of the Southern Root-Knot Nematode and the Root-Lesion Nematode on Alfalfa

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The southern root-knot nematode [Meloidogyne incognita (Kofoid and White) Chitwood] (SRKN) and the root-lesion nematode [Pratylenchus scribneri (Steiner)] (RLN) have overlapping geographic, environmental, and host ranges. The objective of this study is to examine SRKN and RLN interactions on alfalfa using individual and combined inoculations.

The individual SRKN evaluation was inoculated with 5000 SRKN eggs per plant. The individual RLN evaluation was inoculated with 200 RLN per plant. The combined nematode evaluation was inoculated with both 5000 SRKN eggs and 200 RLN. Alfalfa seedlings were inoculated at the unifoliate stage. Approximately 50 conetainers of each entry were inoculated and 20 conetainers of each entry were left as noninoculated checks.

Ninety days after inoculation, cultivar root weights were measured in all three evaluations. Cultivars in the individual SRKN evaluation and the combined evaluation were also scored based on the number of root galls present. Root lesion nematode density per gram of root was measured in the individual RLN evaluations.

Root weights were expressed as a percentage of the mean root weight of the noninoculated check. This proportion was chosen because significant differences among non-inoculated cultivar root weights were found.

'Moapa 69' was used as the SRKN resistant check. 'Lahontan' and 'Caliverde 65' were used as the SRKN susceptible check. 'MNGRN-4' and 'MNGRN-16' were used as the RLN resistant check and 'Baker' as the RLN susceptible check.

The individual SRKN evaluation showed significant differences between resistant and susceptible checks for gall score and root weight. Root weight and gall score were found to have a significant negative correlation.

The individual RLN evaluation showed significant differences between resistant and susceptible checks for root weight. Root-lesion nematodes extracted from cultivar roots were highest in the susceptible check and lowest in the resistant check, although the means were not significantly different.

In the combined nematode evaluation, significant differences for gall score and root weight were found. The combined evaluation, unlike the individual SRKN evaluation, did not show a significant correlation between gall score and root weight. This suggests that in combined nematode inoculations, both the SRKN and the RLN are involved in the reduction of cultivar root weights.

Preliminary Results of a Survey on the Distribution of Plant-Parasitic Nematodes in Australian Alfalfa Fields

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A nematode survey of the occurrence of plant parasitic nematodes associated with alfalfa is being conducted in Australia. The presence of the common nematodes Ditylenchus dipsaci and Meloidogyne spp. and fifteen other genera has been recorded in Australian alfalfa previously from a limited number of samples (Khair, 1987). The wide range of species reported in other countries (Griffin, 1984) and their presence on other agricultural crops in Australia suggested that a wide range of plant parasitic nematodes may be associated also with alfalfa in Australia. This survey is the first systematic national survey of alfalfa fields in Australia.

Alfalfa samples, consisting of soil as well as plant material (roots and shoots) were taken as cores from major alfalfa producing areas of Australia. Each sample of soil was mixed by gentle stirring and 200 g placed on a Whitehead tray. Plant material was finely chopped prior to placement onto Whitehead trays. Nematodes were concentrated with a bank of sieves (38 μm) and observed microscopically.

Ten genera of nematodes were detected in twenty-nine samples analysed to the present (Table 1).

Localities	Monto	Booubyjan	Mudtapilly R.S.	Aberdeen	Tamworth	Muswellbrook	Mudgee	Cowra	Nambrok	Bealiba	Langhorne Ck.	Meningie	Longford	Campbelltown	Ross	Swansea
No. of Samples	3	2	2	1	1	1	1	1	1	1	4	4	1	2	1	3
Pratylenchus	1	0	0	1	1	1	1	1	0	0	0	0	1	2	1	1
Meloidogyne	2	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1
Criconemella	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Paratylenchus	0	1	1	0	0	1	0	0	1	0	1	0	0	1	1	0
Ditylenchus dipsaci	0	0	1	1	0	1	1	1	0	1	4	1	0	1	0	0
Scutellonema	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Tylenchorhynchus	0	0	0	1	1	1	0	1	0	0	0	0	0	1	0	3
Tylenchus	0	0	0	0	0	0	1	0	0	1	0	0	1	1	1	3
Paratrichodorus	0	2	1	0	0	0	0	0	0	0	2	3	0	1	0	0
Xiphenema	1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0

Table 1: Incidence of plant-parasitic nematodes in Australian alfalfa fields.

Pratylenchus sp. and Ditylenchus dipsaci were the most common nematodes in the survey (40% of samples observed). Although Paratylenchus sp. was present at various sites across Australia it was not observed in a recent survey of South African alfalfa fields which detected 18 genera (Marais, 1990). All other genera in this survey were also recorded in South Africa.

Aphelenchoides ritzemabosi was also detected at a number of sites. Although the presence of A. ritzemabosi on lucerne was reported 30 years ago (Grundbacher and Stanford, 1962) only recently has its potential as a pathogen been seriously considered (Williams et al., 1992).

Assessment of pathogenicity of each species, including Aphelenchoides ritzemabosi, will determine which nematode resistance/tolerance genes are to be screened for and eventually integrated into new or existing breeding programs.

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Identification of Thrips-Injured Sympton and the Control of Alfalfa Thrips

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Thrips is one wide spread pest injuring the alfalfa and cause more production decrease than any other pests. Although the thrips causes damage every year, yet it has't drawn enough attention from the people, for its injured sympton is difficult to identify, and often be confused with the virus disease. Thus, experiments were conducted in 1988 ot make correct sympton identification and to develop with different varieties of pestcides for thrips control.

1.The Thrips-Injured Symptons of the Alfalfa: There are altogether three races of thrips causing damage in the Beijing area, Cdentothrips lati (Halidag), Haplothrips chinese Priesher, and Aeolothrips sp. The thrips causes damage by sucking from the epidermis of the alfalfa. The sympton appears as white stripes or circular speckles, the white tipped leaves may also occur or even the whole plant die if damage is serious. The thrips also sucks from the buds and flowers causing no seed-bearing or incomplete seed-filling.

2.The Difference Between the Thrips-Injured Symptons and the Virus Disease Symptons: The virus disease mainly infects the inner tissue parts of the alfalfa, several branches or even the whole plant could be infected. All the infected leaves have the sympton of mosaic chlorosis and uneven leaf surface fulled with dark green bumps and light green holes. sometimes curly leaves may happen, but never shows any speckles. Othermore, the virus disease causes the most damage in April to May, less after the June, because of the rising temperature. Whereas, the thrips causes most damage in June or so, because it reproduces most quickly in the high temperature. At this time, not only the leaves are damaged, but most of the leaf tips are also dried.

3.The Controlling Methords: Spraying the pestcides is the effective way, yet the approciate time and the pestcides varieties must be stressed. A. The Spraying Time: The first speaying of the pestcide should be made in the early of June, when most of the new leaves appear after the first cutting. Afterwards, the pestcide should be sprayed three times consecutively with the seperate of ten days. In the early of July, when the new leaves of the second cutting appear, the pestcide is sprayed again, then after ten days, another spraying should be made.Thus, five spraying is made in the whole year. By this way, the damage of the thrips could be controlled, and increase the production significantly.The production of the fresh weiget with the pestcide spraying is 5.8 tons more than the control in the second cutting,4.5 tons more than the control in the third cutting, 5.4 tons more than the control in the fouth cutting.The total production from the second to the fouth cutting is 15.7 tons more than the control. B. The Varieties and the Concentrations of the Pestcides Sprayed: The results indicate that the mixture of fenvalerate and fenitrothim has the excellent efficiency of the thrips control, with the concentration of 0.02%, and the amount of 54 ml per hectare. It also has good result by spraying the fenitrothion alone with the concentration of 0.025%.

Effect of harvest frequency and root pathogens on survival and yield of two alfalfa lines in Saskatchewan, 1986-90

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An experiment was initiated in 1985 at two locations in Saskatchewan to examine the interaction of 1) two alfalfa lines, 2) harvest frequency, 3) potassium fertilization and 4) inoculation with root pathogens on survival and forage yield of alfalfa. Harvest and inoculation treatments were initiated in 1986 and continued until the tests were terminated in 1989-90. A third site, where the potassium treatment was not included, was also examined. Differences in stand density, persistence and vigor between S-7312 (selected for resistance to Coprinus psychromorbidus) and Beaver were small but important, with S-7312 generally out-performing Beaver. Harvest at 10% bloom (2-3 cuts) did not result in consistent reductions in numbers of plants, stand density or plant food reserves (total non-structural carbohydrates) relative to a single-cut treatment. However, frequent harvests often had a significantly greater impact on Beaver than on S-7312. Frequent harvest occasionally reduced spring vigor and first-cut yield the following spring, but mean total yields for the frequent harvests were 43% higher than for the single-cut treatment (5.3 vs. 3.7 T ha^{-1}). Potassium fertility and inoculation with pathogens had minimal impact on plant survival and yield. Near infrared assessment of total non-structural carbohydrates was accurate, rapid and inexpensive relative to chemical estimates or etiolated growth.

The pattern of crown rot symptom initiation and spread were similar at all sites. Lesions were observed initially at the base of senescing stems and spread laterally into the crown and vertically into the tap root. Lesions were observed in the fall of 1986, but severity was low. By the fall of 1987, incidence was near 100% at all sites and severity had increased markedly. Harvest frequency and potassium fertility did not effect crown rot incidence or severity at any location.

ALFALFA RESEARCH IN ARGENTINA

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Approximately 4.5 to 5 million ha of alfalfa are grown annually in Argentina, mostly located in the Pampean Region. Fall dormancy groups 6 to 9 are widely preferred whereas a relatively reduced area in the South is planted with more dormant types. The performance of foreign cultivars has been quite variable according to the results of 20 years of variety tests.

There are a few unique pests and management practices that should be taken into account when breeding alfalfas for Argentina. In terms of diseases, besides PRR, FW, and AN, the persistence of the crop is also affectd by "corky root rot" (Xylaria sp) and the crown and root rot complex (several fungi). Nematodes and BW are not major problems. Regarding insects, in addition to PA, BAA, and SAA, the crop is also affected by cowpea aphid (Aphis craccivora), several species of Lepidoptera, and larvae of several curculios (Coleoptera). In terms of crop management and utilization, the widely accepted practice of grazing alfalfa implies that some degree of tolerance to trampling is needed. Bloat is also a very significant problem.

In 1970, INTA established the Alfalfa Program to improve the productivity and persistence of the crop. At the present time, the research conducted in many locations encompasses the following main areas and objectives:

* Breeding - To develop cultivars with multiple pest resistance by laboratory and field selection.
* Weed Control - To integrate chemical and alternative management control practices with an ecological criteria.
* Insect Control - To develop an integrated management system for aphids and Lepidoptera species.
* Management - To develop technologies for optimum alfalfa use under grazing, including cultivar evaluation, appropriate management for different dormancy groups and for alfalfa in pure stands or consociated with perennial grasses.
* Seed Production - To develop and promote adapted technologies for an efficient seed production emphasizing irrigation management and native pollinators.
* Alfalfa Cultivar Evaluation Trials Network - To determine the potential yield and persistence of domestic and foreign cultivars and experimental lines under cutting.

There is also a number of new projects recently started:

- Development of alfalfa populations with low initial rate of digestion using the nylon bag technique to attenuate bloat.
- Introduction of the Bt gene into the alfalfa genome (transgenic alfalfa) for the control of Lepidoptera.
- Characterization and evaluation of Argentine alfalfa germplasm.
- Development of nondormant alfalfa populations with a branched root system for the control of curculios (Coleoptera).

Minutes of NCR-138 Alfalfa Diseases Committee Meeting
Held in Conjunction with the
33rd North American Alfalfa Improvement Conference
Atlanta, Georgia
June 14-18, 1992

NCR-138 Committee Members:

P. C. Vincelli, Chair, Kentucky*
L. H. Rhodes, Secretary, Ohio*
G. E. Ham, Admin. Advisor, Kansas*
C. Gabriel, CSRS Rep., Wash., D.C.
C. L. Campbell, North Carolina
C. R. Grau, Wisconsin*
H. W. Kirby, Illinois*
F. W. Nutter, Iowa*
D. L. Stuteville, Kansas*
J. A. Thies, Minnesota*
J. Volenec, Indiana*
J. E. Watkins, Nebraska

*Present at the 1992 meeting

Collaborators Present:

A. Gotlieb, Vermont
S. Nygaard, W-L Research
M. McCaslin, Forage Genetics
D. Brown, Forage Genetics
J. Edmunds, Pioneer
C. Fox, Northrup King
F. Gray, Wyoming
J. Tofte, ABI
S. Stratton, FFR Coop.
N. Altier, Minnesota
G. Hoard, Pioneer
S. Hafez, Idaho
D. Miller, ABI
D. Miller, Cal/West
B. Pennypacker, Pennsylvania
D. Skinner, USDA-ARS (KS)
M. Dickman, Nebraska
D. Samac, Minnesota
P. Gauraud, France
R. Michaud, Canada
K. Dalkin, Pioneer
J. Elgin, USDA-ARS (MD)
K. Leath, USDA-ARS (PA)
R. Pratt, USDA-ARS (MS)
D. Viands, New York
T. Wilson, FSRC
J. Caddel, Oklahoma
M. Wiese, USDA-CSRS
L. Reible, Dairyland Res.
J. Moutray, ABI

Chair Paul Vincelli called the meeting to order on June 15, 1992 at 1:30 p.m. Nine members of NCR-138 and 30 collaborators were present. M. Dickman, substituting for J. Watkins, represented Nebraska. M. Wiese, substituting for C. Gabriel, represented CSRS.

A. Approval of Minutes:

P. Vincelli distributed minutes of the last meeting of NCR-138 and asked for corrections or additions. J. Thies noted that the state report for Minnesota should be corrected to read: "Of 70 samples tested, 42 had Pratylenchus spp." A motion was made by C. Grau to approve the minutes as changed, seconded by W. Kirby. Motion approved.

B. Comments of the Administrative Advisor:

G. Ham announced that NCR-138 had been approved for an additional 4 years. The current extension will run until Sept. 30, 1996. NCR committees are now approved for 4 years rather than 3. There were no problems with the project write-up. Dr. Ham noted the good attendance at the present meeting and indicated that with the present level of attendance and cooperative effort, there should be no problem in further extending NCR-138. However, the current downsizing in Ag. Experiment stations will put pressure on continuation of regional projects. He pointed out the

importance of documenting participation in the NAAIC and CAIC meeting by members of NCR-138, as well as participation of collaborators at the NCR-138 meeting. (See section D). NCR-138 will receive a mid-term review by department heads.

Two new members of NCR-138 were recognized: Forrest Nutter, Iowa State University representing Iowa, and Jeff Volenec, Purdue University, representing Indiana.

G. Ham discussed membership in NCR-138. F. Gray, University of Wyoming indicated that he had not been able to get approval for membership in NCR-138. M. Wiese noted that there were no restrictions on committee membership with respect to geographical boundaries. G. Ham indicated that it was only necessary to have the experiment station director write a letter in support of membership.

C. Comments of the USDA-CSRS Representative:
Dr. Maury Wiese, representing USDA-CSRS, spoke to the group about CSRS activities and programs. Dr. Wiese is the chair of the Department of Plant Pathology at the University of Idaho and is currently on sabbatical at CSRS. The new CSRS reps include O. W. Barnett, K. Barker, M. Wiese, and A. Kelman, who is serving as chief scientist for the National Research Initiative. Hatch funds have been stabilized and are approximately the same as last year. IPM grant funds will total approximately 1 million in each of 4 regions. Overall, the success rate of the competitive grants program is about 20%.

D. Participation of NCR-138 Committee Members in the 33rd North American Alfalfa Improvement Conference (NAAIC):
A total of 11 papers or posters were presented by NCR-138 committee members at the present meeting of NAAIC. C. Grau is immediate past chairman and L. Rhodes is current vice-chairman of the Central Alfalfa Improvement Conference.

E. Old Business:
P. Vincelli gave an update on the directory of NCR-138 members and collaborators. A new directory is being prepared and will be distributed in 1992.

F. New Business:
Nominations. The floor was opened for nominations for secretary of NCR-138 for 1993 (to serve as chair in 1994). F. Nutter was nominated by C. Grau and elected unanimously.

Future meeting locations. NCR-138 will meet with the CAIC in Lincoln, NE, June 20-23, 1993. A motion was made by C. Grau to meet with the NAAIC in Guelph, Ontario in 1994. Seconded by F. Nutter. Motion approved.

F. State Reports:
P. Vincelli noted that because of the high interest in Sclerotinia crown and stem rot (SCSR), Dr. Bob Pratt, USDA-ARS and Mississippi State, had been invited to speak to the NCR-138 group. Dr. Pratt is well-known for his work on Sclerotinia in alfalfa and other forage legumes.

Mississippi - R. Pratt. SCSR is a severe problem of clover and other legumes in the South. Stands are lost over winter and early spring. Sclerotia are formed on colonized plants and serve as oversummering structures. No further disease development occurs until apothecia emerge in fall/winter. Foliar clipping in the fall has proven effective in controlling SCSR. Presumably infected foliage is removed before the fungus can move into the crown. SCSR development may also be restricted by band seeding rather than broadcast. Planting in rows denies the fungus the ability to spread 360 degrees. Wide row spacings work best but yields are reduced. Intermediate row spacings are a compromise between disease development and forage yield. The stem lesion assay is being used to select for resistance. Progeny from plants selected by this method have lower disease severity indices than parent populations.

Ohio - L. Rhodes. Screening of alfalfa P.I.'s for Sclerotinia resistance using a seedling assay is continuing. 112 P.I.'s and 254 PVP's were evaluated this year. Some fairly striking differences in disease development were seen in both PI and PVP collections. A field trial to determine levels of Sclerotinia resistance was established in 1991. Differences in stand density between varieties were seen in Spring 1992. Plots treated with 4 applications of Ronilan to control Sclerotinia had the highest percent stand and yield in the first cutting.

Iowa - F. Nutter. A research program has been initiated in epidemiology of foliar diseases. Preliminary work is being done to determine which pathogens are present. During the wet spring of 1991, spring black stem accounted for approximately 90% of leaf lesions; by June, SBS accounted for less than 1% of lesions. Chlorothalonil sprays are being used to obtain different levels of disease for a yield loss model. In 1991 yield loss was >30%, but less than 5% this year. A crop scan radiometer is being used to assess disease severity. This appears to be more precise than visual estimates. In a survey for AMV, greater than 50% incidence was found at one site.

Minnesota - J. Thies. Research on Pythium is continuing. N. Altier has isolated Pythium at 12, 18 and 24 C and done pathogenicity tests at the same temperatures. Pythium isolates are being identified to species. Field evaluations for lesion nematode resistance are continuing. P. penetrans causes root necrosis and stand thinning. Soil populations of lesion nematode in field tests are 200-300/100cc soil. Sainfoin, birdsfoot trefoil, and clovers seem to be more susceptible than alfalfa.

Illinois - W. Kirby. 1991 was relatively free of disease problems. Ergot was a problem on grasses invading alfalfa stands thinned by heaving and winterkill. Sclerotinia occurred in west-central Illinois. Sclerotinia appears as a problem only about once in 3 years. The use of mineral oil and sodium bicarbonate for foliar disease control is being investigated.

Nebraska - M. Dickman. No alfalfa disease problems apparent this year, with the exception of some spring black stem during cool weather in the spring. Current alfalfa research is concentrated on host-pathogen physiology of anthracnose. An isolate of Colletrotrichum from C. Grau, apparently C. trifolii, is virulent on Saranac AR and may be a new race.

Wyoming - F. Gray. Recently toured agricultural areas in France. Verticillium Wilt is a major problem there. Stem nematode is also a significant problem. Sclerotinia was found causing problems in alfalfa-ryegrass mixtures, possibly because of matted grass. Presently working on interaction of chyrsanthemum foliar nematode and stem nematode. Both nematodes survive in anabiotic state in dead tissue. Verticillium wilt study recently published in Plant Disease. A stand decline problem in Verticillium-resistant varieties on a ranch in Wyoming is currently being investigated.

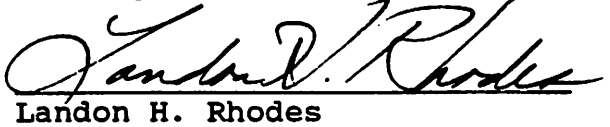
Wisconsin - C. Grau. The branched root characteristic appears highly related to Aphanomyces resistance. Selection for the branching root habit tends to improve resistance to Aphanomyces. Has obtained a single outstanding plant from a flooded field and is currently investigating flooding tolerance in progeny from this plant. Studies with metalaxyl have shown that this compound may predispose plants to non-target pathogens.

Kansas - D. Skinner. Studies on downy mildew are continuing. Presently using molecular markers to identify resistant plants. Also using RAPD techniques to study phylogeny of Stemphylium. Results indicate that there are 5 different Stemphylium species causing leaf spot in alfalfa. Molecular technique agrees closely with morphological identification. Also using PCR to look at differences in potato leafhopper populations. Results indicate many different genotypes of potato leafhopper, which may be why genetic resistance has been a difficult trait to breed for. In a host range study of Uromyces striatus it was found that an isolate from alfalfa will infect many other legume species.

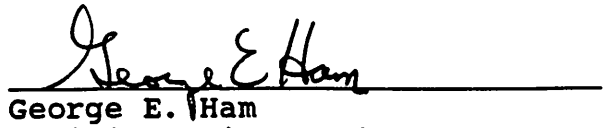
Kansas - D. Stuteville. L. Johnson is continuing work on plant transformation. Attempting to move protease inhibitor genes into alfalfa. No expression as yet. C. Chaisrisook has examined numerous Stemphylium isolates using the RAPD technique. S. alfalfae was the species identified most frequently. Only one isolate of S. botryosum was found. Downy mildew is still a serious problem in Kansas. Evaluation of P.I.'s to identify resistance are continuing.

Kentucky - P. Vincelli. A soil bioassay is being used to determine presence of Aphanomyces. A. euteiches was found in 50-60% of soil samples throughout the state. Nodules are primary sites of infection. Feeder root necrosis is a common symptom. Aph-resistant varieties had higher yields in field trials, but this may be overall breeding progress since only newer varieties have Aphanomyces resistance. Apothecia of Sclerotinia were found to be present over a 10-week period. A control program of fall cutting plus fungicide application was studied. The unsprayed control had 10% surviving plants, Ronilan (2 lb.) plus a fall cut had 5.8%, and Ronilan (4 lb) had 64.4%

Meeting Adjourned.



Landon H. Rhodes
Secretary



George E. Ham
Administrative Advisor

Current Status of Alfalfa Pathology in Illinois

H. Walker Kirby
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University of Illinois
Urbana, IL.

Alfalfa acxreage continues to increase throughout much of southern and northern Illinois. Demand for high quality hay around the Chicago area also continues to increase, with premium prices being paid for high quality, high nutrient forage. The Department of Agronomy operstes a forage testing and evaluation program to advise producers on the best selections for each area of the state because of the differences in both climate and disease potential from northern to southern Illinois.

Sclerotinia crown and stem rot continues to be a serious first-year production problem for many growers in western central to southern Illinois. Although epidemics of this disease appear in limited areas and are further limited by weather patterns, many producers still lose sizable acreages. Because of these factors, as well as the lack of varietal resistance, a fungicide plus a monitoring program would offer the best approach. Development of such a system would permit an integrated approach to management while limiting fungicide applications.

Foliar diseases continue as the major pathogen complex throughout much of the Illinois production areas. Southern Illinois, in particular, offers a more favorable environment for the development of leafspot problems and leaf and quality losses. Currently, there are no highly effective fungicides available, although some growers are conducting their own liomited testing programs. This year, small plots have been established to determine if biologically acceptable materials can be substituted for commercial fungicides to reduce leafspot losses. Pathogens such as Phoma medicaginis, Stemphylium botryosum, Leptosphaerulina spp., and Colletotrichum spp. will be monitored following applications of both commercial fungicides and organic alternatives. The products to be tested include mancozeb (standard), copper hydroxide (labelled), sodium bicarbonate, and a refined horticultural oil. The latter two materials have previously demonstrated activity comparable to commercial fungicides in both greenhouse and field trials for selected foliar pathogens of ornamental crops without the common hazards associated with these products.

1992 NCR-138 (Alfalfa Diseases)
Report for Iowa

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Project Personnel:
S.A.A. Rizvi, Plant Pathology Post Doctoral Research Associate
P.M. Shultz, Plant Pathology Research Associate
D.R. Buxton, USDA, ARS, Agronomist
J.J. Obrycki, Iowa State University, Entomologist

I. Seasonality of Foliar Diseases in 1991

The seasonality of foliar plant pathogens and their affect on alfalfa yield and quality was studied by establishing field plot experiments in a north-south transect at four research farms in Iowa: Ames, Ankeny, Knoxville, and Chariton. Nine different leaf-spotting fungi and one bacterial leaf pathogen were found to occur at all four locations. Spring black stem, caused by Phoma medicaginis, was the predominant foliar pathogen early in the growing season and accounted for 80% or more of the leaf-spot lesions that were present on alfalfa leaves at the time of the first weekly sampling date . The 1991 spring was one of the wettest on record and percent defoliation values caused by spring black exceeded 70% in 1991. Yield reductions attributable to spring black stem were 35.1% at Ankeny and 19.7% at Chariton for the first harvest date.

By mid to late June, spring black stem epidemics were largely replaced by common leaf spot (Pseudopeziza medicaginis) and Leptosphaerulina leafspot (Leptosphaerulina briosiana) with peak periods of Pseudopeziza occurring before 18 July. Cercospora medicaginis (summer black stem and leafspot) epidemics began in early June and disease severity continued to increase until the third harvest (mid-August). These foliar pathogens reduced hay yield by 31.7% in Ankeny and 2.6% at Chariton at the second harvest and 25.8% and 29.7% at the third harvests, respectively. Other pathogens occurring in low frequencies were Colletotrichum dematium and bacterial leafspot (early spring), and Leptotrochila medicaginis, and Uromyces striatus. These experiments are being repeated in 1992.

II. Effect of Alfalfa Foliar Pathogens on Yield and Quality.

Fungicide concentrations (chlorothalonil) ranging from 0 to 1.26 kg/ha a.i. were applied every 10 days to field plots located in Ankeny and Chariton, Iowa as a means to create a range of disease intensity values. Regression models relating disease intensity (X) to hay quanity and quality (Y) are presently being developed. Although foliar pathogens significantly reduced quantity of yield, quality was not affected. Yield loss experiments are being repeated in 1992.

III. Incidence of Alfalfa Mosaic Virus.

The incidence of alfalfa mosaic virus (AMV) was determined by local lesion assay and by serological confirmations using ELISA. AMV incidence was extremely high at the Ames (50%) and Ankeny (36%) field locations while only about 1% of the plants sampled at the Chariton and Knoxville locations were infected with AMV in 1991.

Acknowledgement: This research was supported, in part, by a grant awarded to the ISU Alfalfa IPM Issue Team by the Leopold Center for Sustainable Agriculture and by funds provided by the Iowa State Agriculture and Home Economics Experiment Station, Ames, IA. Appreciation is also expressed to John Hill, ISU Plant Virologist, for help with the AMV study, and Dwayne Buxton, USDA-ARS Agronomist, for processing quality samples.

1992 NCR-138 (Alfalfa Diseases) Report

Donald L. Stuteville
Department of Plant Pathology
Kansas State University
Manhattan, KS 66506

Personnel: Chulee Chaisrisook, Grad. Res. Asst., Plant Pathology
Xiongfei Ding, Grad. Res. Asst., Plant Pathology
Lowell B. Johnson, Plant Disease Physiologist
Daniel Z. Skinner, Alfalfa Geneticist
Edgar L. Sorensen, Alfalfa Geneticist ("retired")
Donald L. Stuteville, Forage Crop Pathologist

Transformation (LBJ and XD). Research is being initiated to attempt alfalfa transformation with genes for several proteinase inhibitors in an effort to enhance pest resistance.

Molecular Markers (DZS, DLS). A trait-based method of associating molecular markers with traits of interest is being developed. Initial studies are centered on resistance to downy mildew and random amplified polymorphic DNA (RAPD) markers. A computer program has been written which compiles frequencies of occurrence of individual fragments and combinations of two or three fragments. Combinations of three fragments have been found in significantly higher proportions in resistant plants than in susceptible plants, and vice-versa. However, absolute association of fragment combinations with resistance has not been established.

Stemphylium (CC, DZS, DLS). Principal component analysis of percentages of RAPD fragments in common and phylogenetic analysis have corroborated Simmons' conclusion that at least five Stemphylium species attack alfalfa. Studies with pulse field gel electrophoresis to compare electrophoretic karyotypes of these species have been initiated.

Alfalfa Rust (DZS, DLS). A study of the host range of a rust fungus from alfalfa continues. Known hosts currently include 50 species including all Medicago species tested, most Trigonella species tested, several Melilotus and Trifolium species and one Astragulas species. The study is being expanded to include more distantly related taxa.

Downy Mildew (DLS, CC). In cooperation with the USDA Regional Plant Introduction Station, Pullman, WA, we are continuing to evaluate selected PI accessions for resistance to isolate I-7 and I-8 of Peronospora trifoliorum.

Development of Multi Pest Resistant Germplasms (ELS, DLS, DZS, and others). Alfalfa germplasms resistant to disease and insect pests important in the Great Plains are continuing to be developed cooperatively by the USDA and KAES. KS219 has recently been registered (Crop Sci. 32:502). KS220 has been released by the USDA and KAES. KS221, KS222, and KS223 have been approved for release by the KAES. KS220, derived from broad-based NC-83-2; KS221, derived from B1C-7; KS222, derived from 'Anchor' alfalfa; and KS223, derived from a 25-clone synthetic (KS63), all have resistance to anthracnose (race 1), bacterial wilt, downy mildew, Fusarium wilt, pea aphid and spotted alfalfa aphid. Additionally, KS220 has resistance to Verticillium wilt, Phytophthora root rot and the blue alfalfa aphid. KS221 also has resistance to Verticillium wilt and Phytophthora root rot. KS223 also has resistance to the blue alfalfa aphid and the 25 clones comprising parental KS63 evidenced little feeding in field plots severely damaged by alfalfa weevil larvae.

NCR-138 REPORT ON ALFALFA PATHOLOGY RESEARCH IN KENTUCKY

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Department of Agronomy
University of Kentucky
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Long-term experiments have been initiated on two diseases of alfalfa in Kentucky: Aphanomyces root rot, caused by *Aphanomyces euteiches*; and Sclerotinia crown and stem rot (SCSR), a very destructive disease of fall seedings caused by *Sclerotinia trifoliorum*.

An ongoing soil survey indicates that *A. euteiches* is widespread in Kentucky alfalfa fields. Trials are being conducted to test the hypothesis that resistance to *A. euteiches* will enhance alfalfa yields in Kentucky soils naturally infested with the pathogen. A trial was established using fourteen varieties with different levels of resistance to *A. euteiches*. All varieties had ratings of MR or higher to other important diseases. The trial was seeded 18 Apr 91 and harvested on 26 Jun, 30 Jul, and 5 Sep 91. Yield data were pooled into two groups, susceptible (S rating) or resistant (MR, R, or HR rating) to *A. euteiches*. Yields were not significantly different between the two groups in the first and third cutting, but the resistant varieties collectively outyielded ($P<0.01$) the susceptible varieties by 10.1% in the second cutting. At this point, it is unclear whether the observed yield increase is due to genes for resistance to *A. euteiches* or to other genetic factors. These studies are being expanded to include another site naturally infested with *A. euteiches* as well as sites free of the pathogen.

In a previous experiment, complete control of SCSR was achieved in a fall-seeded alfalfa stand with three applications of vinclozolin fungicide. Current research with SCSR is directed at testing the hypothesis that the disease can be controlled under Kentucky conditions with a single fungicide application by seeding a variety with a low level of resistance and taking a freezedown harvest. In an experiment seeded 2 Sep 91, severe stand loss from SCSR occurred in plots of Hi-Phy alfalfa (low levels of resistance) that had been treated with vinclozolin (1.12 kg ai/ha) at apothecial emergence (29 Oct 91) and harvested on 14 Nov 91. Preliminary stand counts indicated that 94% of crowns in the treated plots had been killed, as compared to 90% in untreated plots and 36% in plots treated twice with vinclozolin (2.24 kg ai/ha). Surviving plants in the trial were killed this spring by a combination of repeated freeze injury and heavy feeding by alfalfa weevils; thus, complete yield and stand data were not collected. However, the preliminary results raise questions about the feasibility of controlling SCSR under Kentucky conditions with only one fungicide application. Future experiments will test whether postponing fungicide application until after the freezedown harvest will enhance the level of disease control.

NCR-138 Committee (Alfalfa Diseases) Report

Judy A. Thies

USDA/ARS and University of Minnesota
Co-investigators: D.K. Barnes, N.A. Altier,
L.A. Wanschura, and D.M. Smith

Evaluations for disease and nematode resistances.

Approximately 150 alfalfa cultivars and experimental populations were evaluated for resistances to bacterial wilt, Fusarium wilt, and Phythophthora root rot at Rosemount and St. Paul, MN. The 1989 test to screen and evaluate public and proprietary alfalfa germplasms for field resistance to root-lesion nematode, Pratylenchus penetrans, was was dug at Grand Rapids, MN in September, 1991. Scientists representing eight alfalfa seed companies made selections from their respective experimental alfalfa populations that had been entered in the test.

Evaluation of alfalfa for seedling resistance to Pythium spp.

A culture plate method was developed to evaluate alfalfa germplasm for resistance to Pythium seed rot and seedling damping-off. Fourteen isolates of Pythium spp. from MN and WI alfalfa field soils were evaluated for pathogenicity to the Beltsville International Composite-7 (BIC-7) alfalfa population at three temperatures: 12, 18, and 24 C. Two hundred fifty-five North American alfalfa cultivars also were evaluated for seedling resistance using three isolates differing in pathogenicity and morphology. There was a significant interaction between isolates and temperature. Percent Resistant Plants (PRP) in the BIC-7 population varied ($P < 0.05$) for different isolates from 0 - 50%. Alfalfa cultivars varied for PRP from 0-25% and for ASI from 3.25 to 5.00 for different isolates.

1991 NCR-138 REPORT FOR OHIO

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Plant Introduction Evaluations. 113 PI's and 254 PVP's were evaluated for Resistance to Sclerotinia using a standard growth chamber seedling assay. Substantial differences in disease development were noted within both PI and PVP collections.

Sclerotinia Field Variety Trial. A field trial with 18 entries was established in August, 1991, at Columbus. Ten companies submitted seed of 13 varieties or experimental lines for entry in the trial. An additional 5 entries were selected as checks, including Armor alfalfa sprayed 4 times with Ronilan for absolute control of Sclerotinia. Curibaya (very similar to PI 172188), was included as a resistant check, but proved to be non-dormant and was severly thinned over the winter.

Alfalfa stands throughout the plot area were relatively uniform throughout the fall. The first apothecia were observed on Nov. 22, 1991.

Plants in all varieties broke dormancy following a week of warm weather in early March and suffered frost injury when temperatures fell to 16 F in mid-March. When re-growth resumed in April, many plants were observed to have been killed, and some stands were greatly reduced. All dead plants examined at this time had sclerotia on crowns at or below the ground line. Stand depletion did not occur in Ronilan-sprayed check plots, further indicating that losses were due to Sclerotinia rather than cold temperature injury. Data on number of infected plants per plot were taken May 5-8, 1992; however, these data may not accurately reflect true levels of disease incidence since earlier-killed plants were not recorded at this time. Percent stand and first-harvest yield were highest in the Ronilan-sprayed control.

Ronilan Residue Analysis. Alfalfa was sprayed with Ronilan (vinclozolin) on Nov. 20, 1990 (at apothecium emergence). Fresh forage and hay samples from first cutting alfalfa (May 20, 1991) were submitted for residue analysis. No detectable residues of Ronilan were found (detection limit=0.5 ppm).

Phytophthora Root Rot. Studies on control of Phytophthora with Apron and Ridomil were continued in 1991. Ridomil (1 pint/A) or Apron plus Ridomil significantly increased plant counts at 6 weeks. Yields were extremely low due to drought and intense leafhopper pressure and were not affected by Apron or Ridomil treatment.

PENNSYLVANIA
1992 NCR-138 (Alfalfa Diseases) Report

USDA, ARS Pasture Laboratory and Penn State University
University Park, PA 16802

PERSONNEL: Y. Guevera, A.A. Hower, D.P. Knievel, K.T. Leath, F.L. Lukezic, B.W. Pennypacker, R. Rodriguez and W. Sackett

Fusarium oxysporum - Sitona hispidulus Interactions (AAH, KTL): This research on the activity of F. oxysporum in feeding sites on roots of alfalfa has been completed. Main conclusions are: 1) F. oxysporum is the most common pathogen in these sites, followed by F. solani and other F. spp. 2) Many isolates of F. oxysporum from feeding sites cause Fusarium wilt of alfalfa; 3) Lateral spread of fungi from feeding sites is slow; and 4) the combination of curculio eggs and F. oxysporum produced more severe Fusarium wilt than was caused by the fungus alone.

Resistance Factors in Alfalfa to Verticillium albo-atrum (BWP, KTL, DPK): A histological study was completed to determine the fate of V.a.a. hyphae in resistant plants. Following stubble inoculation, the fungus moved down through the stubble, through the crown, and up into the stem xylem. This spread took longer in resistant plants than in susceptible plants. Many of the responses to infection were similar in both resistant and susceptible plants. These include coating of vessel walls, plugging of pits, occlusion of vessels, occurrence of atypically small but mature vessels, hypertrophy of xylem parenchyma, and crushing of infected vessels. In resistant plants, hyphae were encased by a "lignituber" type of formation, which did not occur in susceptible plants. Also, photosynthesis was maintained in infected leaves of resistant plants but not in comparable leaves from susceptible plants.

Survival of Biocontrol Bacteria on Alfalfa Leaves (FLL, YG, KTL, WS): Bacteria have potential as biocontrol agents against foliar pathogens of alfalfa. Nutrients, osmoprotectants and UV-light protectants were added to bacterial suspensions to determine if survival of the bacteria on alfalfa leaf surfaces would be enhanced. Candidate bacteria were Rifampicin resistant, and reisolation was done on a selective medium. Greatest survivability of the test bacterium (Pseudomonas putida, PSU831) was obtained with a combination of adjuvants: King's B broth plus dextrose, betaine and folic acid. SEM Studies showed that bacteria survived on both leaf surfaces, but were most numerous on the lower surface. Sites most commonly occupied by bacteria were around the bases of and on trichomes, along leaf veins, around stomates, and in crevices caused by cell wall junctures.

Evaluation of PI Lines for Foliar Disease Resistance (KTL): One hundred and fifty accessions from the alfalfa PI Core Collection are under evaluation for reaction to Lepto, common, Phoma and Stemphylium leaf spots. This should complete evaluation of the core collection for these diseases.

Wisconsin NCR-138 Report for 1992

Craig R. Grau
Department of Plant Pathology
University of Wisconsin-Madison
Madison, WI 53706

Improvement of Alfalfa Populations for Wet Soil Environments
Selection for a branching root type has resulted in a increased frequency of plants resistant to A. euteiches, but not to P. medicaginis. A single plant was selected at the Marshfield Research Station which expressed a superior phenotype for the ability to grow in a highly water saturated soil environment in November, 1991. This plant is being evaluated as a source of flood tolerance. Alfalfa populations were exposed to intensive selection pressure for winter survival at the Marshfield Research Station during the Winter of 1991-92. Alfalfa populations that are selected to branching root type, flood tolerance and winter hardiness are being evaluated for resistance to A. euteiches, P. medicaginis, and Rhizoctonia spp.

Resistance to Race 2 Phenotypes of A. euteiches
The frequency of race 2 resistant plants has been increased by selection in five alfalfa populations that trace back to the PI's 468018 (Canada), 439006 (Syria), 434593 (Japan), 206572 (Greece), and 464781 (Turkey). Efforts are in progress to transfer these sources of race 2 resistance identified in the PI's into alfalfa germplasm adapted to the North Central USA.

Evaluation of PVP Varieties for Resistance to A. euteiches
Alfalfa varieties were evaluated for resistance to A. euteiches (race 1) as part of program to characterize alfalfa varieties for the PVP program. Of 254 varieties evaluated, 190 were susceptible, 51 had low resistance, 10 were moderately resistant, 3 were resistant and none were rated as highly resistant.

Variability for Virulence within Aphanomyces euteiches
Race 1 of the alfalfa strain of A. euteiches was detected in soils collected in Wisconsin, Kentucky, Minnesota and Pennsylvania. However, race 2 phenotypes, virulent to WAPH-1, were recovered from soil from the Marshfield Research Station.

Effect of Metalaxyl Fungicide on Alfalfa Establishment
The number of plants per m^2 was greater in plots planted with seed treated with metalaxyl compared to plots seeded with untreated seed. Forage yield was not improved with metalaxyl in the seeding year.

PUBLICATIONS:

Grau, C. R., Nygaard, S. L., Arny, D. C., and Delwiche, P. A. 1991. Comparison of methods to evaluate alfalfa cultivars for reaction to Verticillium albo-atrum. Plant Disease 75:82-85.

Tofte, J. E., Smith, R. R., and Grau, C. R. 1991. Selection for resistance to Aphanomyces euteiches in red clover. Crop Sci. 31:1141-1144.

Grau, C. R., Muehlchen, A. M., Tofte, J. A., and Smith, R. R. 1991. Variation in virulence in Aphanomyces euteiches. Plant Disease 75:1153-56.

Tofte, J. E., Smith, R. R., and Grau, C. R. 1992. Reaction of red clover to Aphanomyces euteiches. Plant Disease 76:39-42.

Grau, C. R. 1992. Registration of WAPH-1 alfalfa germplasm with resistance to Aphanomyces root rot. Crop Sci. 32:287-288.

WYOMING

1992 NCR-138 (Alfalfa Diseases) Report
Submitted by F. A. Gray, Plant, Soil, & Insect Sciences Department
University of Wyoming, Laramie 82071

Chrysanthemum foliar nematode (F. A. Gray, J. L. Williams & T.E. Wilson)

Distribution -The CFN (*Aphelenchoides ritzema-bosi*) appears to be widespread throughout the western region, occurring in the same stem bud tissues with the alfalfa stem nematode (ASN). The occurrence of both phytoparasitic nematodes in symptomed, alfalfa stem bud tissue, has been found in France (Dr. George Caubel, Nematologist, I.N.R.A., Le Rheu, France) and Australia (Dr. Iand Kaehne, Plant Breeder, Dept. Agriculture & Fisheries, Adelaide, Australia), indicating a possible worldwide occurrence of these two nematodes in alfalfa.

Resistance - Our recent studies indicate cultivars having resistance to the ASN also have resistance to the CFN. Resistance most likely is the result of selection for ASN resistance in fields where both nematodes were present. Continuation of plant selection using pure cultures of *D. dipsaci* may lead to a reduction in CFN resistance and an eventual reduction in the performance of ASN-resistant cultivars under field conditions.

DISEASE X WEED INTERACTION (F. A. Gray, S. D. Miller & M. Shiek)

A study was established in northcentral Wyoming to determine the interaction of diseases and weeds on yield and plant populations in alfalfa. Data were collected for 5 years. Phytophthora root rot (PRR), Verticillium wilt (VW) and the alfalfa stem nematode (ASN) were present. Both Phytophthora root rot and Verticillium wilt caused significant stand reductions in resistant and susceptible alfalfa cultivars while weeds failed to reduce plant stands. Alfalfa yields decreased while weed yields increased over the 5-year period, regardless of treatment. As disease severity increased, weeds became more prevalent especially in the non-pesticide treated plots.

VERTICILLIUM WILT

Harvest management-grazing study - (F. A. Gray & D. W. Koch)

This is a long term study to determine the effect of late harvest and grazing of aftermath on plant stand and yield in VW-resistant cultivars under a natural disease infestation. Plots have a natural infestation of VW. Both late harvesting and grazing appear to accelerate stand decline. These additional stresses may cause increased death of VW-infected plants. This study will be continued through 1995.

Recent Publications:

1. M. S. Page, F. A. Gray and R. L. Hossfeld. 1991. Infection status of healthy-appearing alfalfa plants of resistant cultivars in Verticillium wilt-infested fields. Proceedings of the 7th Western Alfalfa Improvement Conference.
2. Williams, J. L. 1991. Etiology of the alfalfa stem and chrysanthemum foliar nematodes in alfalfa. M. S. Thesis, University of Wyoming, Laramie. 71 pp.
3. M. S. Page, F. A. Gray, D. E. Legg, and W. G. Kearl. 1992. Economic impact and management of Verticillium wilt on irrigated alfalfa hay production in Wyoming. Plant Dis. 76:504-508.
4. F. A. Gray, M. S. Page, D. E. Legg, and R. L. Hossfeld. 1992. Evaluating alfalfa for field resistance to Verticillium wilt. Journal of Prod. Agric. 5:273-278.

NAAIC President's Report

Jim B. Moutray
Director of Forage Research
ABI Alfalfa
Ames, IA 50010

It has been an honor to serve as President during the 1990-92 period. During the past two years the Executive Committee met three times and spent most of its time planning the 33rd Conference and helping to organize the committees responsible for conducting the activities of the NAAIC.

The 33rd Conference was well-attended and ran smoothly. The three-day format (rather than four) seemed to work well, with comments in favor of continuing three-day meetings.

Financially, the Conference is in the best shape it has ever been, due mainly to sound planning by the 32nd location committee, headed by Richard Peaden. Our financial condition has also been improved by sustaining memberships through the efforts of the Finance Committee.

The new program to support graduate travel with funds generated by sustaining memberships went well.

I want to personally express a great big "Thanks" to all committee chairpersons and committee members for their work and support during the past two years.

The affairs of NAAIC are in good hands for the next period with Gary Bauchan assuming the Presidency.

Report of the Executive Secretary - June 14, 1992

Membership

NAAIC membership as of June 4, 1992 is shown below:

North American		424
United States	388	
Canada	36	
Non-North American		138
Total Membership		562

In addition, 63 libraries receive the NAAIC Proceedings

NAAIC Publications

Report of 32nd NAAIC

Distributed approx. 425 of the 475 printed - 200 in the U.S., 30 in Canada, 125 overseas, and 63 libraries (37 in the North America and 26 overseas).

1991 Alfalfa Scientists Directory

Distributed approx. 385 of 500 printed - 230 in the U.S., 30 in Canada, and 125 overseas. There are 383 North American listings and 125 Non-North American listings.

Standard Tests Notebook

Sold 205 of 500 copies printed.

Financial Statement

Reporting Period August 11, 1990 - June 14, 1992

Beginning balance		$ 6,701.53
Income during period		18,336.08
Total funds available		$ 25,037.61
Expenses during period	-	$ 11,313.95
Balance June 14, 1992		$ 13,723.66

Respectfully Submitted
James H. Elgin, Jr.
Exec. Secretary, NAAIC
June 14, 1992

FINANCE COMMITTEE REPORT

The current financial condition of the North American Alfalfa Improvement Conference is good. The account balance as of June 4, 1992 was $13,983.66 ($10,233.66 in the general fund, $4,000 in the sustaining membership fund). Estimated income for the 1992-94 period is $6,500; estimated expenses for the same period are $5,200, leaving a projected balance for the 34th NAAIC in 1994 of $15,273.00. This does not include additional Sustaining Membership monies which may be collected.

A proposal for establishment of a Sustaining Membership Fund was presented and passed at the 32nd NAAIC in Pasco, WA. Funds collected would be used to pay for travel of honorary award recipients who are without travel funding support and to supplement graduate student travel to future NAAIC meetings. The Finance Committee was charged with soliciting monies for this program and for selecting graduate student travel grant recipients. The Executive Secretary was responsible for collection of monies and subsequent disbursements to award/grant recipients. A letter soliciting contributions to the Sustaining Membership Fund was mailed in March 1992 to 53 companies in Canada and the United States representing seed, inoculant, chemical, and equipment companies active in the alfalfa industry. To date, we have sixteen sustaining members, for a total of $4,000 in the Sustaining Membership Fund.

An application form for graduate student travel grants was developed and included in the first conference mailing for the 33rd NAAIC in Atlanta. A number of award applications were received and four graduate student travel grants were awarded to the 33rd NAAIC in Atlanta. In addition, one honorary membership travel grant was awarded, leaving approximately $2,000 in the Sustaining Member Fund for use at the 34th NAAIC. The Finance Committee will continue to solicit Sustaining Memberships in the coming months, with the hope that a greater number of travel grants will be available for the 34th NAAIC.

Gary Bauchan
Dave Miller
Jim Moutray
Dan Undersander
Mike Peterson, Chairman

Sustaining Members of the 33rd NAAIC
June 14-18, 1993, Atlanta, GA

ABI Alfalfa
Rt. 3
Ames, IA 50010

Allied Seed Co-op, Inc.
1917 E. Fargo Avenue
Nampa, ID 83687

Brett-Young Seeds, Ltd.
P.O. Box 99
St. Norbert Postal Station
Winnipeg, Manitoba
Canada R3V 1L5

Cal/West Seeds
P.O. Box 1428
Woodland, CA 95695

Cargill Hybrid Seeds
P.O. Box 5645
Minneapolis, MN 55440

CelPril Industries, Inc.
251 Oak Street
Manteca, CA 95336

Dairyland Seed Co., Inc.
P.O. Box 958
3570 Hwy. H
West Bend, WI 53095

FFR Cooperative
4112 E. State Road 225
West Lafayette, IN 47906

Forage Genetics
N5292 Gills Coulee Rd., South
West Salem, WI 54669

Gustafson, Inc.
P.O. Box 660065
Dallas, TX 75266-0065

ICI Seeds, Inc.
6945 Vista Drive
West Des Moines, IA 50265

Liphatech
3101 West Custer Avenue
Milwaukee, WI 53209

Northrup King Company
317 - 330th Street
Stanton, MN 55081

Pickseed Canada, Inc.
Box 126
Richmond Hill, Ontario
Canada L4C 4X9

Pioneer Hi-Bred Int'l., Inc.
7305 N.W. 62nd Avenue
P.O. Box 287
Johnston, IA 50131

W-L Research, Inc.
8701 Hwy. 14
Evansville, WI 53536-9593

NAAIC Executive Committee Meeting Minutes
Kansas City Airport Marriot
October 8, 1990

Gary Bauchan, Joe Bouton, Mark McCaslin, Richard Peaden and Don Viands were in attendance. Jim Moutray sent his regrets for missing the meeting due to a family emergency.

The meeting was called to order by Gary Bauchan. Gary also read the minutes from the last Executive Committee Meeting and gave the Executive Secretary's Finance Report. Our current balance is unchanged since last meeting, $6,701.53. This balance will change once a complete accounting of the cost of the 32nd NAAIC proceedings is available.

The Standard Test Bulletin has been delayed due to a loss of personnel at NK. Cheryl Fox had previously been able to get help at NK in putting together this new revision. Cheryl has agreed to look into other alternatives.

Gary Bauchan reported that the Finance Committee will begin solicitation of Sustaining Memberships in 1991. Paul Sun, Mike Peterson and Dave Miller will be involved in this effort.

Richard Peaden reported on the 32nd NAAIC as follows:

a) There were 162 registrants, 45 of which paid a late registration fee. There were 16 guests in attendance.
b) Eight companies donated $50 each to help defray banquet costs and eight vendors paid $100 each for booth setup.
c) $27,977 was taken in from registration and donations. Total expenses, prior to the publishing and distribution of the proceedings, were $18,010.
d) It was agreed that a letter of appreciation from the NAAIC be sent to those companies, Pioneer and WL Research, that provided meals on the post conference tour.

Gary Bauchan reported that the conference proceedings would include all paper abstracts from both the NAAIC and Forage Insect Workshop (max 1 page each). It was agreed that a brief summary from each state in the NCR-138 session would also be included. Reports from the regional improvement conferences and the following NAAIC committees would also be in the proceedings: Available Breeding Lines Committee (Michaud) and the Finance Committee (Sun).

There was lengthy discussion on the format of future NAAIC meetings including the following alternatives:

a) 3 day meeting with limit on the number of oral papers and more emphasis on poster sessions.
b) 4 day meeting with emphasis on oral papers, no concurrent sessions.
c) 3 day meeting with emphasis on oral papers, some concurrent sessions.

The following schedule was suggested for the 33rd NAAIC:
Monday - full day paper session
Tuesday - 1/2 day tour, 1/2 day paper session
Wednesday - 1/2 day paper session, 1/2 day business meeting with banquet Wednesday evening.

Concurrent sessions for biotechnology/production and NCR-138/Forage Insect Workshop were suggested. Joe Bouton suggested a 1/2 day tour at the Forage/Livestock Research Station in Eatenton, GA and a post conference tour to Athens. Joe has looked into potential facilities near Atlanta for the conference.

Gary Bauchan provided a recommended schedule in preparing for ht 33rd NAAIC meeting. This agenda is attached.

There being no further business, the meeting was closed.

NAAIC Executive Committtee Meeting Minutes
St. Paul, Minnestoa
November 16, 1991

Gary Bauchan, Joe Bouton, Jim Elgin, Cheryl Fox, Mark McCaslin, Jim Moutray, Don Viands and Tim Woodward were in attendance.

The meeting was called to order by Jim Moutray. Mark McCaslin read the minutes from the last Executive Committee Meeting. Jim Elgin gave the Executive Secretary's Finance report. A written summary was distributed summarizing our expenses and income since the last report. Jim stated that our current balance, $10,652.57, is higher than normal due to the larger than expected income from our annual meeting in Pasco. We can expect additional income from several new NAAIC publications currently in distribution: 32nd NAAIC Report, the 1991 Alfalfa Scientist Directory, and the new Standard Tests Notebook. Mailing of NAAIC publications and communications has been handled by the Executive Secretary and USDA/ARS. Jim reported that this will not be possible after 1996 and suggested that we begin to explore other options.

Joe Bouton reported on local arrangements for the upcoming 33rd NAAIC in Atlanta. The conference will be held at the Atlanta Holiday Inn, near the airport. The cost will be $60 per room per night, with free meeting rooms. Joe says that the hotel has excellent access to local restaurants. He has planned for registration of about 175 persons. Three meeting rooms have been reserved to cover concurrent sessions, poster sessions and possibly vendor displays. A reception is planned for Sunday evening, June 14. Oral and paper sessions will fill the day on Monday followed by a recreational event on Monday night. The scientific presentations will resume on Tuesday morning after short regional conference breakfast meetings, beginning at 7:30AM. On Tuesday afternoon we will take a field trip to Eatenton, GA. The scientific presentations begin again on Wednesday morning and are interupted at 11:00AM, for the NAAIC annual meeting. The papers will resume again after lunch. The banquet will be held Wednesday evening. A post conference tour to southern Georgia, including Tipton, will take place on Thursday.

There was ample discussion concerning the above schedule. A consensus of the committee agreed to balance oral and paper presentations as needed and to try and to limit the business meeting to one hour by streamlining committee reports. Decisions concerning concurrent sessions will be discussed as the program begins to take shape. It was also decided that lunch on Monday would be on our own and that the banquet costs would be part of registration. The banquet format will be similar to last year with the local arrangements committee

to arrange for a MC and for entertainment. There was discussion about having vendors at the meeting. It was decided that vendors will be invited only if there is enough room to accomodate them without interfering with the scientific presentations.

Jim Elgin reported on the honors and awards committee for Ken Leath, who was absent. The R.R. Hill Achievement Award will presented for the first time this year. This award recognizes recent achievement in alfalfa improvement. There will be up to 6 honorary memberships given this year (1% of membership). Jim Moutray asked Ken Leath, Mike Peterson (chair of the Finance Committee) and Cheryl Fox (chair of the Industry Committee) to caucus to discuss providing money for conference travel for new honorary members. The executive committee agreed in principle to transfer up to $3000 from the general fund for this purpose (motion by Elgin, second by McCaslin, motion passed). Jim Moutray suggested listing all honorary members in future proceedings.

Jim Moutray distributed a list of all committee chairs along with minutes from each committee meeting in Pasco.

There was active discussion on how to handle revisions to the new Standard Tests Notebook. It was decided that the membership would be notified of any changes as they occur and sent an order form to purchase the new or revised testing procedures as they become available (motion by Elgin, second by McCaslin, motion passed).

The Executive Committee will urge the PVP Office accept the changes in the PVP application recommended by the Minimum Distance Committee (motion by Woodward, second by McCaslin, motion passed).

The 1994 NAAIC meeting is scheduled for Guelph, Ontario. Gary Bauchan stated that Guelph had recently turned down an EFIC meeting and suggested that we double check on the willingness of Guelph to host the 34th NAAIC meeting. Quebec City was suggested as a likely backup.

There being no further business, Jim Moutry adjourned the meeting.

NAAIC Executive Committe Minutes
Atlanta, Georgia
June 14, 1992

Gary Bauchan, Joe Bouton, Jim Elgin, Cheryl Fox, Mark McCaslin, Jim Moutray, Mike Peterson, Don Viands, and Tim Woodward were in attendance.

The meeting was called to order by Jim Moutray. Mark McCaslin reviewed the minutes from the last meeting. Mike Peterson gave a report from the Finance Committee. He reported that the new sustaining member program attracted 15 companies, with contributions totaling $3750. A list of the sustaining members is attached. A total of 53 companies were contacted in the letter of solicitation. Jim Elgin displayed a sample certificate to be sent to sustaining members - various comments were made concerning the design of the certificate. A final version will now be prepared for distribution. The sustaining member fund supplied $3000 for student and honorary member travel ($2000 for John Baylor and $250 for each of four student speakers). Mike Peterson suggested increasing the student travel allotment from $250 to $500 per student.

Joe Bouton reported that registration was down somewhat from previous years, with 130 registrants as of Sunday afternoon. The details of various local arrangements were discussed including seating at the banquet and the opening of the conference.

Jim Elgin gave a detailed financial report and reviewed the status of several recent NAAIC publications. A summary of this report from our executive secretary is attached.

Tim Woodward brought up the issue of isolation distances required in the production of seed of varieties with patented genes. The issue concerns the likelyhood of a neighboring field picking up stray pollen carrying a patented gene. There was considerable conversation about this and related issues. Mark McCaslin suggested that this and other regulatory type issues be handled by a new standing committee, the Regulatory Issues Committee. Since the current Minimum Distance Committe represents a good sampling of public and private breeders, it was further recommended to combine the charges of these committees under the auspices of the new standing committee. After some discussion this suggestion was adopted by the executive committee by unanimous vote.

Don Viands reported that the nominations commitee was prepared to nominate Real Michaud as incoming secretary. This met with very favorable reaction from those present.

Gary Bauchan reported for the locations committee that the next NAAIC meeting would be held in Guelph, Ontario in late June or mid July, 1994. Dr. Steve Bowley will chair the local arrangements committee.

Cheryl Fox gave a report for the standardization committee. She reported on the status of two new standard tests on forage quality and ML expression. A 10 location forage quality test was established this year to help furnish information on expected values for check varieties. There was some discussion on check varieties in general and a need to update checks regularly. It was decided that this issue should be addressed by the industry committee.

There being no further business, Jim Moutray adjourned the meeting.

Minutes from the 9th Eastern Forage Improvement Conference
Business Meeting held at The University of Prince Edward Island, Charlottetown, P.E.I.

Thursday, June 28, 1991

The meeting was called to order at 4:55 p.m. by chairman, Bert Christie.

Norm Lawson made a motion to waive the reading of the minutes from the last EFIC meeting due to their distribution to the membership. The motion was adopted.

The balance in the EFIC account remains the same as the last meeting, $342.00

Gary Bauchan made an announcement concerning the 32nd North American Alfalfa Improvement Conference which was held on August 19 through the 24, 1990 in Pasco, Washington. There were 180 participants who attended the meeting. The meeting consisted of 2 1/2 days of oral and poster presentations, 1/2 day tour, and concluded with an awards banquet.

The 33rd North American Alfalfa Improvement Conference will be held on June 14 through 18, 1992 in Atlanta, Georgia. For additional information contact Dr. Joseph Bouton, University of Georgia, Agronomy Dept. 3111 Plant Sciences Building, Athens, Georgia 30602 (404) 542-2461. The tentative schedule is as follows: Sunday - Reception;Monday -all day papers; Tuesday - 1/2 day papers & 1/2 day tour + BBQ; Wednesday - 1/2 day papers & 1/2 day business meetings + banquet; Thursday - Post Conference Tour (University of Georgia).

A motion was made by Norm Lawson that the Eastern Forage Improvement Conference is in favor and is willing to lend scientific support to the bid for the 1997 International Grassland Congress to be held in Ottawa, Ontario, Canada. Bill Murphy seconded the motion and the motion was passed.

The following report was submitted from the resolutions committee, consisting of Dr. John Bubar (chairman) and Dr. Julie Hanson:

> Whereas The Agriculture Canada Research Station, Prince Edward Island Department of Agriculture, and the Atlantic Veterinary College, University of Prince Edward Island acted as hosts of the Ninth Eastern Forage Improvement Conference under the leadership of Dr. Bert Christie (chairman) and his staff including; Y. Papadopoulos (program), T. Kunelius (symposium), M. Suzuki (posters), P. Narasimhalu (reception), B. Dickson (finance), L. Halliday (Barbecue), A. Kielly (tour), Clint McLean and Bill Thomas (post-conference tours) and M. McNiven (liason, AVC), with contributions from Atlantic Livestock Feed Initiative, Co-Op Atlantic, Potash & Phosphate Institute of Canada, and the University of Prince Edward Island; and whereas participants provided a wide range of technical papers and posters on forages that application and improvement of forages for our region and freely discussed their work. Be it resolved that we, the participants in the conference, extend our sincere appreciation to these individuals ad organizations for making the conference in Prince Edward Island so productive and enjoyable.

This motion was seconded and unanimously approved.

The following report was given at the barbecue on Thursday evening from the EFIC Graduate Student Competition Committee composed of Don Viands (chairman), Clinton McLean and Art McElroy. First Place: James Johnston (University of Guelph) for his paper on Sward height in grazing management; Second Place: Bill Thomas (Nova Scotia Agricultural College, Truro, N.S.) for his paper entitled "Cell wall composition and digestibility of stems from six alfalfa varieties differing in resistance to lodging;" and Third Place: Nancy McLean (Nova Scotia Agricultural College, Truro, N.S.) for her paper "In vitro regeneration in red clover." The EFIC Graduate Student Competition was sponsored by the Potash & Phosphate Institute of Canada. In total $500 in awards were presented, $250 for first place, $150 for second place and $100 for third place. Congratulations to all the graduate students who presented papers at the conference.

The nominations committee, consisting of Gary Bauchan (chairman), Bruce Coulman, and Don Viands, proposed the following slate of officers for the 1991 EFIC:

Chairman: Al Gotleib, University of Vermont, Burlington, VT.
Vice-chairman: Yousf Popadapoulus, Agriculture Canada, Nappan, Nova Scotia

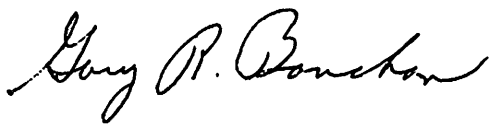
There were no further nominations from the floor and the above executive was approved unanimously.

An invitation from Ken Leath was presented to hold the next (10th) EFIC at Penn State University as a joint meeting with the Northeastern Branch of the American Society of Agronomy. The tentative for the next EFIC is June 20 - 25, 1993. Mark your calendars!

An invitation from Art McElroy was presented to hold the eleventh EFIC at Agriculture Canada in Ottawa, Ontario.

The outgoing Chairman, Dr. Bert Christie, transferred the office to Dr. Bill Murphy, in the absence of vice-chairman Al Gotleib, who thanked Dr. Christie for his efforts during the meetings. Dr. Murphy adjourned the meeting with the absence of no further business at 5:25 p.m.

Respectfully submitted by:



Gary R. Bauchan
Permanent Secretary
Eastern Forage Improvement Conference

REGIONAL CONFERENCE REPORT
FOR THE
CENTRAL ALFALFA IMPROVEMENT CONFERENCE

The 22nd CAIC was held June 17-19, 1991, at Ames, Iowa, at the Holiday Inn Gateway Center. Craig R. Grau was program chair and presided over the conference. The conference was attended by 76 participants. There were 14 oral presentations and 10 posters presented. Conference participants were given a tour of the Pioneer Hi-Bred International, Inc. research facilities at Johnston, Iowa; AgriPro Biosciences research facilities at Napier, Iowa; the Iowa State University forage research facilities near Ames, Iowa. The NCR-138 (Alfalfa Diseases) met in conjunction with the CAIC. Six reports were presented to all participants interested in alfalfa pathology.

Officers of the CAIC for 1992-93 are: Tim Woodward (Pioneer Hi-Bred International, Inc.), President; Lanny Rhodes (Ohio State University), Vice-President; Cheryl Fox (Northrup King), Secretary. The 23rd CAIC is scheduled for June 20-22, 1993 at Lincoln, Nebraska. Bruce Anderson, Steve Danielson and John Watkins, at the University of Nebraska, are responsible for local arrangements.

John Caddel continues to take responsibility for the CAIC Variety Test Reports with approximately 100 reports sent to recipients. The report has also been made available on computer disks.

Submitted by,

W. T. W. Woodward
CAIC President

1992 Central Alfalfa Improvement Conference Business Meeting

June 17, 1992

Chairman Tim Woodward called the meeting to order at 7:30 a.m. on June 17, 1992. The CAIC business meeting was held in conjunction with the 1992 North American Alfalfa Improvement Conference at Atlanta, GA. Chairman Tim Woodward asked for corrections to the minutes of the 1991 meeting. The minutes were accepted as reported in the 1991 CAIC proceedings.

Old Business- John Caddel reported that the cost of printing and distributing the CAIC Alfalfa Variety Trial Evaluation Summary was covered by Alfalfa industry contributions. The distribution of computer disks with the information from some state trials was started in 1990 and was considered very useful by those receiving disks. The practice will be continued and expanded to include all of the states if possible. A request form for individuals wanting to remain on the mailing list was included in the summary. Anyone who did not return the request and still wishes to receive the summary was asked to contact John Caddel. The conference thanked John for continuing to provide the summary.

Conference members were advised that extensive information on the alfalfa core collection is now available including information on morphological traits, pest reaction, quality traits and geographic origin. Data on reaction to Pithium resistance will be added in the near future. Information on specific traits for the core collection is available on the GRIN system.

Length of the abstracts was discussed and it was decided that one page was adequate. Longer reports for NCR-138 could be condensed to fit the one page format.

New Business- The locations committee announced that the 1993 CAIC will be hosted by the University of Nebraska in Lincoln, NE on June 20-22, 1993.

Mark McCaslin suggested the possibility of inviting other NCR or NC committees to meet with the CAIC. There is a bypass protein group, NCR189, chaired by T. J. Klopftenstein, Animal Science Dept., University of NE. Lanny Rhodes will contact that group to discuss a possible joint meeting or look into the possibility of having one or two invited speakers address the meeting on some aspect of forage quality.

The meeting was adjourned at 8:00 a.m.

Respectfully submitted by: Cheryl C. Fox, Secretary, Central Alfalfa Improvement Conference

WESTERN ALFALFA IMPROVEMENT CONFERENCE

MINUTES 1992 BUSINESS MEETING
HOLIDAY INN - ATLANTA, GEORGIA

Minutes of the business meeting held on June 17, 1992, at the Holiday Inn, Atlanta, Georgia.

The meeting was called to order by Cliff Currier and Don Miller in Dave Evan's absence.

Election of a new non-moving treasurer was discussed due to the resignation of John Haight. After a brief discussion, Don Brown was elected to the office of non-moving Treasurer. Don Brown and Don Miller agreed to oversee the WAIC checking account deposited in Nampa, Idaho. A letter of appreciation to John Haight for his service to the Society and the industry was drafted.

Don Miller gave the Treasurer report regarding the meeting in Nampa, Idaho (1991). Cost of the meeting, including publishing of the proceedings was $2,706.37, leaving a surplus of $408.63. A check for this amount was given to Don Brown for deposit in the WAIC account.

Minutes of the last meeting were handed out and read by Don Miller. The new by-laws presented at the 1991 meeting were discussed again, and it was suggested that a few minor changes were still needed. These changes were discussed and approved by the members. Jim Elgin suggested an additional change in the by-laws may be needed to address what happens to the WAIC monies in the event the organization is disbanded. He suggested that the amendment could be similar to the National by-laws and said he would send a copy to Don Miller for review at the next meeting.

It was proposed that these revisions should be sent to the members by mail before the 1993 meeting.

WESTERN LOCATION YIELD REPORT COMMITTEE: Cliff Currier was instructed by President Dave Evans to thank Bob Romanko (in his absence) for his efforts in publishing the WAIC yield report and requested that he continue on for an additional year. Bob Romanko agreed to continue but he noted that the cost of the publication may need to increase to $1300. Cheryl Fox was again asked to solicit these funds.

Bob suggested that the data should be submitted on computer disks for ease of publication, however, other members thought

we should continue to accept the data in any form to insure publication.

Dave Evans, by letter, suggested that a long term procedure should be set up to insure the publication of the yield data when Bob Romanko steps down. He suggested two possibilities: 1.) to have the Vice-President assume responsibility during tenure of office, and, 2.) to seek a "non-moving" Editor akin to our Treasurer as the need arises. These suggestions were presented to the members but no decision was made.

INTER-REGIONAL VARIETY TRIAL COMMITTEE: Larry Teuber noted that this information has not been published yet.

Larry Satterlee presented the information he collected regarding the availability and cost of university yield trials in the West. He noted tht cost of entries in trials ranged from $90 - $1500/entry. Examples of costs were given as follows:

Arizona will have trials but cost is not known-

California - $400/location	Nevada - $350/location
Colorado - $400/location	New Mex.-$300/location
Idaho - $1500/location	Oregon - $175/year
Montana - $200/location	Utah - $90/location

NEW BUSINESS: Dr. Tom Mc Coy confirmed that Montana State University was planning to host the 1993 meeting of WAIC at Bozeman, Montana. He suggested that the meeting be held on August 5 - 6, 1993.

Officers for the 1993 meeting will be as follows:
Dave Evan - President
Don Miller - Vice President
Ray Ditterline - Secretary
Don Brown - Treasurer (non moving)

Respectfully,

Donald Miller

DON MILLER
WAIC Secretary

DRM:jk

NAAIC INDUSTRY LIAISON COMMITTEE REPORT

Full or partial support for the following publications was arranged by the committee:

- 1990 and 1991 WAIC Variety Trials Report
- 1990 and 1991 CAIC Variety Trials Report

The contributions and number of contributors has been adequate to cover costs in the last two years.

A cooperative study on quality factors, particularly check cultivars to be used for the proposed quality test, has been initiated. The following companies and public institutions have contributed materials and/or agreed to plant the experiment and collect quality samples:

- ABI
- Cornell University
- Great Plains Research
- Montana State University
- Northrup King Co.
- Pioneer Hi-Bred International Inc.
- Purdue University
- University of Georgia
- University of Kentucky
- University of Minnesota
- Vista Research
- W-L Research Inc.

The following varieties were selected for the study:

- P5432
- Vernal
- WL-322HQ
- Oneida VR
- VS820 (MF)
- NK91795 (MF)
- Arrow
- Cimarron

The samples will all be analyzed by Craig Sheaffer at the University of MN.

The question of seed production of bio-engineered varieties and what if any modifications are necessary to the present system is under discussion.

David Jessen was selected as new chairperson for the committee.

Committee: Cheryl Fox - Chair, Dave Jessen, Lauren Johnson, Mark McCaslin, Mike Peterson

Report of the Committee on Available Breeding Lines of Alfalfa

Nineteen alfalfa germplasms have been released subsequent to the 1990 North American Alfalfa Improvement Conference, (Table 1). Eighteen of the germplasms have been registered in Crop Science. The committee encourages that seed samples of all germplasms released be submitted to the National Seed Storage Laboratory (NSSL) at Fort Collins for long term storage. In addition to the germplasm release notice, the committee encourages that all germplasms be registered and published in Crop Science.

At the 1984 conference, the Committee recommended an updated version of the publication "Improved Breeding Lines of Alfalfa" (ARM-W-5/ Sept:1978). It was decided by the current committee that the summary of already published information would be a duplication and is not needed.

In the proceedings of the 1990 NAAIC meeting (Prosser, WA) tables were presented to show breeding lines, cultivars approved by the National Certified Alfalfa Variety Review Board (1962-1982) and discontinued cultivars that have not been stored at the NSSL. The committee urges all responsible people to see that all cultivars and germplasms be registered and a seed sample stored at the NSSL.

The committee encourages the storage of standard reference alfalfa cultivars or germplasms be stored at the Plant Introduction Station in Pullman, WA. A standard reference alfalfa cultivar or germplasm has been defined as one that was first described as representing or possessing a unique characteristic (s) or a unique genetic origin, a wide acceptance for its use as a standard for comparison, or one that is of historical significance to the alfalfa industry. A list of these cultivars and germplasms is listed in the 1990 NAAIC proceedings. Cultivars and germplasms fitting one or more of these categories should be submitted. Seed lots submitted for storage should be well documented: where grown, germination, year of production, generation of synthesis and certifying agency.

Committee: Cliff Currier - Chairman

Table 1 - Germplasm releases subsequent to the 1990 report of the Committee on Available Breeding Lines : NAAIC

Germplasm	Crop Science & PI#	Contact	Description
W2xiso-1	C.S. 31:496 PI 542967	E.T. Bingham U. of Wisconsin	W2xiso-1 is a diploid population isogenic to tetraploid W4xiso-1 cultivated alfalfa developed by chromosome manipulations. Isogenic W2xiso-1 was developed for comparative research on diploids and tetraploids and has not been screened for disease resistance or agronomic traits.
W4xiso-1	C.S. 31:496 PI 542968	E.T. Bingham U. of Wisconsin	W4xiso-1 is a tetraploid population isogenic to diploid W2xiso-1 cultivated alfalfa developed by chromosome manipulations. Isogenic W4xiso-1 was developed for comparative research on diploids and tetraploids and has not been screened for disease resistance or agronomic traits.
REGEN-SY	C.S. 31:1098 PI 537440	E.T. Bingham U. of Wisconsin	Regen-S is a M. sativa type, and Regen-Y is a M. falcata type that is easily transformed with Agrobacterium tumefaciens. One self progeny from each of the two parents was selected based on its regeneration ability and the two selected self progeny were handcrossed to produce Regen-SY.
AZ-90NDC-ST	C.S. 31:1098-9 PI 545592	S.E. Smith U. of Arizona	AZ-90NDC-ST is a broad-based, nondormant, alfalfa germplasm exhibiting superior forage production in greenhouse trials under conditions of moderate NaCl stress. AZ-90NDC-ST was derived from AZ-88NDC, a composite nondormant germplasm.
UC 73	C.S. 32:284 PI 552540	L. Gibbs U. of California El Centro, CA	UC 73 is a nine-clone synthetic germplasm resulting from two cycles of recurrent phenotypic selection for low weevil larvae feeding in San Diego County, California, from an unknown source of nondormant alfalfa.
UC 176	C.S. 32:285 PI 552541	L. Gibbs U. of California El Centro, CA	UC 176 was selected over 15 years to develop resistance to one or more of the following diseases in the low desert of southern California: phytophthora root rot (caused by Phytophthora megasperma Drechs. f. sp. medicaginis T. Kuan & D.C. Erwin); rhizoctonia root canker (caused by Rhizoctonia solani T. Kühn); fusarium wilt [caused by Fusarium oxysporum Schlechtend. f. sp. medicaginis (J.L. Weimer) W.C. Snyder & H.N. Hans.]; the physiological, low-oxygen-tension problem commonly called scald and blue alfalfa aphid (Acyrthosiphon kondoi Shinji) during their development. Approximately 50 to 80% of the parentage of the germplasms trace to 'UC Salton', 'UC Cargo', and 'CUF 101'. The remainder is unknown. All seed was produced by open-pollination.
UC 196	C.S. 32:285 PI 552544	L. Gibbs U. of California El Centro, CA	UC 196 was selected over 15 years to develop resistance to one or more of the following diseases in the low desert of southern California: phytophthora root rot (caused by Phytophthora megasperma Drechs. f. sp. medicaginis T. Kuan & D.C. Erwin); rhizoctonia root canker (caused by Rhizoctonia solani T. Kühn); fusarium wilt [caused by Fusarium oxysporum Schlechtend. f. sp. medicaginis (J.L. Weimer) W.C. Snyder & H.N. Hans.]; the physiological, low-oxygen-tension problem commonly called scald and blue alfalfa aphid (Acyrthosiphon kondoi Shinji) during their development. Approximately 50 to 80% of the parentage of the germplasms trace to 'UC Salton', 'UC Cargo', and 'CUF 101'. The remainder is unknown. All seed was produced by open-pollination.

Table 1 - Germplasm releases subsequent to the 1990 report of the Committee on Available Breeding Lines : NAAIC (continued)

Germplasm	Crop Science & PI#	Contact	Description
UC 226	C.S. 32:285 PI 552546	L. Gibbs U. of California El Centro, CA	UC 226 was selected over 15 years to develop resistance to one or more of the following diseases in the low desert of southern California: phytophthora root rot (caused by Phytophthora megasperma Drechs. f. sp. medicaginis T. Kuan & D.C. Erwin); rhizoctonia root canker (caused by Rhizoctonia solani T. Kühn); fusarium wilt [caused by Fusarium oxysporum Schlechtend. f. sp. medicaginis (J.L. Weimer) W.C. Snyder & H.N. Hans.]; the physiological, low-oxygen-tension problem commonly called scald. Approximately 50 to 80% of the parentage of the germplasms trace to 'UC Salton', 'UC Cargo', and 'CUF 101'. The remainder is unknown. All seed was produced by open-pollination.
UC 276	C.S. 32:285 PI 552549	L. Gibbs U. of California El Centro, CA	UC 276 was selected over 15 years to develop resistance to one or more of the following diseases in the low desert of southern California: phytophthora root rot (caused by Phytophthora megasperma Drechs. f. sp. medicaginis T. Kuan & D.C. Erwin); rhizoctonia root canker (caused by Rhizoctonia solani T. Kühn); fusarium wilt [caused by Fusarium oxysporum Schlechtend. f. sp. medicaginis (J.L. Weimer) W.C. Snyder & H.N. Hans.]; the physiological, low-oxygen-tension problem commonly called scald. Approximately 50 to 80% of the parentage of the germplasms trace to 'UC Salton', 'UC Cargo', and 'CUF 101'. The remainder is unknown. All seed was produced by open-pollination.
UC 296	C.S. 32:285 PI 552550	L. Gibbs U. of California El Centro, CA	UC 296 was selected over 15 years to develop resistance to one or more of the following diseases in the low desert of southern California: phytophthora root rot (caused by Phytophthora megasperma Drechs. f. sp. medicaginis T. Kuan & D.C. Erwin); rhizoctonia root canker (caused by Rhizoctonia solani T. Kühn); fusarium wilt [caused by Fusarium oxysporum Schlechtend. f. sp. medicaginis (J.L. Weimer) W.C. Snyder & H.N. Hans.]; the physiological, low-oxygen-tension problem commonly called scald. Approximately 50 to 80% of the parentage of the germplasms trace to 'UC Salton', 'UC Cargo', and 'CUF 101'. The remainder is unknown. All seed was produced by open-pollination.
UC 189	C.S. 32:285-6 PI 552542	L. Gibbs U. of California El Centro, CA	UC 189 is a nondormant germplasm with high resistance to fusarium wilt [Fusarium oxysporum Schlechtend. f. sp. medicaginis (J.L. Weimer) W.C. Snyder & H.N. Hans.], moderate resistance and resistance, respectively, to bacterial wilt [Clavibacter michiganense subsp. insidiosum (McCulloch)], and moderate resistance to phytophthora root rot (Phytophthora megasperma Drechs. f. sp. medicaginis T. Kuan & D.C. Erwin). UC 189 is an 18-plant synthetic resulting from three cycles of recurrent pheonotypic selection for bacterial wilt resistance at St. Paul, MN. CUF 101 served as the base population, and UC 143 and UC 123 were intermediate germplasms.

Table 1 - Germplasm releases subsequent to the 1990 report of the Committee on Available Breeding Lines : NAAIC (continued)

Germplasm	Crop Science & PI#	Contact	Description
UC 231	C.S. 32:285-6 PI 552542	L. Gibbs U. of California El Centro, CA	UC 231 is a nondormant germplasm with high resistance to fusarium wilt [Fusarium oxysporum Schlechtend. f. sp. medicaginis (J.L. Weimer) W.C. Snyder & H.N. Hans.], moderate resistance and resistance, respectively, to bacterial wilt [Clavibacter michiganense subsp. insidiosum (McCulloch)], and moderate resistance to phytophthora root rot (Phytophthora megasperma Drechs. f. sp. medicaginis T. Kuan & D.C. Erwin). UC 231 is a 235-plant synthetic from the second and third cycles of selection for bacterial wilt resistance at St. Paul and traces to CUF 101 (75%) and UC 103 (25%), a population selected from 'UC Salton' in a low, wet area near Blythe, CA.
UC 195	C.S. 32:286 PI 552543	L. Gibbs U. of California El Centro, CA	UC 195 is a very nondormant germplasm with high resistance to the blue alfalfa aphid (Acyrthosiphon kondoi Shinji) and fusarium wilt [caused by Fusarium oxysporum Schlechtend. f. sp. medicaginis (J.L. Weimer) W.C. Snyder & H.N. Hans.] and resistance to phytophthora root rot (caused by Phytophthora megasperma Drechs. f. sp. medicaginis T. Kuan & D.C. Erwin). UC 195 is a 242-clone synthetic germplasm selected for resistance to the blue alfalfa aphid. Its parentage traces to BAA1 (37%), 'CUF101' (33%), 'UC Salton' (28%), and 'Mesa Sirsa' (2%).
UC 222	C.S. 32:286-7 PI 552545	L. Gibbs U. of California El Centro, CA	UC 222 is a very nondormant germplasm with high resistance to the blue alfalfa aphid (Acyrthosiphon kondoi Shinji). UC 222 originated from a wide, very nondormant germplasm base maintained by the University of California alfalfa breeding program at El Centro, CA.
UC 263	C.S. 32:286-7 PI 552548	L. Gibbs U. of California El Centro, CA	UC 263 is a very nondormant germplasm with high resistance to the blue alfalfa aphid (Acyrthosiphon kondoi Shinji). UC 222 originated from a wide, very nondormant germplasm base maintained by the University of California alfalfa breeding program at El Centro, CA.
UC 332	C.S. 32:287 PI 552551	L. Gibbs U. of California El Centro, CA	UC 332 is a very nondormant germplasm with high resistance to fusarium wilt [caused by Fusarium oxysporum Schlechtend. f. sp. medicaginis (J.L. Weimer) W.C. Snyder & H.N. Hans.] and phytophthora root rot (caused by Phytophthora megasperma Drechs f. sp. medicaginis T. Kuan & D.C. Erwin. UC 332 originated from selections obtained from UC 186 (47 clones), UC 193 (23 clones), UC 195 (50 clones), and UC 196 (47 clones), whose parentage traces to 'CUF 101' (44%), 'UC Salton'-type germplasm (39%) and unknown germplasm (17%).
WAPH-1	C.S. 32:287-8 PI 552564	C.R. Grau U. of Wisconsin	WAPH-1 was selected for resistance to aphanomyces root rot caused by Aphanomyces euteiches Drechs. WAPH-1 is a tetraploid (2n = 4x = 32) synthetic population developed from a strain cross of two populations. WAPH-1 traces to cultivars 'Answer', 'Apollo II', 'Armor','Blazer', 'Challenger', 'Drummer', 'G7730', 'Magnum', 'Onieda', 'Trident', and 'Saranac AR'.

Table 1 - Germplasm releases subsequent to the 1990 report of the Committee on Available Breeding Lines : NAAIC (continued).

Germplasm	Crop Science & PI#	Contact	Description
KS 219	C.S. 32:502-3 PI 555667	Dept. of Agronomy Kansas State University	KS 219 resulted from recurrent phenotypic selection for resistance to bacterial wilt (2 cycles), downy mildew (1 cycle), anthracnose (1 cycle), phytophthora root rot (4 cycles), blue alfalfa aphid (3 cycles), pea aphid (1 cycle), and spotted alfalfa aphid (2 cycles). KS 219 was derived from a strain cross (Graham X Riley). Graham is an unknown germplasm of alfalfa that has been grown in Oklahoma for more than 50 years.
KS 220	----	Dept. of Agronomy Kansas State Unversity	KS 220 was derived from NC-83-2. The population was subjected to recurrent phenotypic selection for resistance to anthracnose (2 cycles), bacterial wilt (2 cycles), downy mildew (6 cycles), Fusarium wilt (2 cycles), Phytophthora root rot (6 cycles - 4 in greenhouse and 2 in field), Verticillium wilt (1 cycle), blue alfalfa aphid (4 cycles), pea aphid (5 cycles), and spotted alfalfa aphid (4 cycles).

1992 Alfalfa Crop Advisory Committee Report

Role of the Alfalfa Crop Advisory Committee: The public and private-sector scientists of the Alfalfa Crop Advisory Committee (ACAC) provide leadership and advice on issues pertaining to *Medicago* germplasm. The ACAC has developed and helped implement strategies for germplasm collection, seed increase, and evaluation, and the development of core collections. The overall goal of the ACAC is to insure that the widest range of *Medicago* germplasm resources are available and useful to all potential users. The committee is primarily responsible for administration of the U.S. Plant Introduction (PI) Collection of perennial and annual *Medicago* species. This collection, held at Pullman, WA, contains nearly 2300 accessions of *M. sativa*, 387 accessions of other perennial *Medicago* species, and 2260 accessions of annual *Medicagos*.

Current ACAC Activities: **Collection** of additional perennial *Medicago* germplasm is in progress or planned in five regions (Himalaya of India, Caucasus Mountains of Russia, N. and W. China, S.E. China, and N. Africa and Arabia). **Seed increase** of perennial species within the U.S. PI collection is conducted at Prosser, WA and annual *Medicagos* are increased at Riverside, CA. Through 1990 about 1,750 perennial and 1,150 annual PIs have been increased. A significant proportion of the perennial accessions have been **evaluated** for 54 traits and results included in the GRIN database. **Core collections** will allow more systematic use of the collections and reduce maintenance requirements. A dynamic core collection, containing approximately 200 accessions of *M. sativa*, has been developed using geographical, agronomic, and morphological data. Construction of core collections for annual *Medicagos* (containing about 200 accessions) and *Rhizobium meliloti* are also in progress. In order to better serve germplasm users, the ACAC developed and distributed a **germplasm use questionnaire** to the North American members of the NAAIC. In addition to information on respondent's job responsibilities and employers, the questionnaire asked about past use of PI germplasm and traits that users considered important and about information retrieval on *Medicago* germplasm. (For a more thorough discussion of questionnaire results, see abstract by S. E. Smith in Report of 33rd NAAIC Conference.). The ACAC is also working with the Plant Variety Protection Office to **standardize** the national alfalfa variety database.

Future ACAC activities and goals: (1) Provide leadership and support for the maintenance and evaluation programs headquartered at Pullman, WA, with emphasis on refinement of core collections for perennial and annual *Medicagos*; (2) Promote research on germplasm description and classification, and the on the usefulness of genetic variation in *Medicago*; (3) Identify needs for, and facilitate collection of *Medicago* germplasm; (4) Foster use of *Medicago* germplasm; and (5) Strive to improve cooperation among scientists and organizations involved with *Medicago* germplasm.

ACAC Membership:

Gary R. Bauchan
USDA, Beltsville, MD

Donald K. Barnes
USDA, St. Paul, MN

Richard Berberet
Oklahoma State Univ.

Mark A. Bohning (Ex-officio)
USDA, Beltsville, MD

Joseph H. Bouton
Univ. of Georgia

James H. Elgin, Jr.
USDA, Beltsville, MD

William R. Ellis
Seedbiotics, St. Joseph, MO

Cheryl Fox
Northrup King & Co., Stanton, MN

Walter Graves
Univ. Calif. Coop. Exten.

Richard C. Johnson
USDA, Pullman, WA

Mark McCaslin
Forage Genetics, W. Salem, WI

Thomas J. McCoy
Montana State Univ.

Jim Moutray
ABI, Ames, IA

Richard N. Peaden
USDA, Prosser, WA

Landon H. Rhoades
Ohio State Univ.

Melvin D. Rumbaugh
USDA, Logan, UT

Steven E. Smith (Chairman)
Univ. of Arizona

Dave Stout
USDA, Pullman, WA

Larry R. Teuber
Univ. Calif., Davis

Jeffery J. Volenec
Purdue University

William T. W. Woodward
Pioneer Hi-Bred, Johnston, IA

REPORT OF STANDARD TEST COMMITTEE OF NAAIC - 1992

As a result of the revision and expansion of the bulletin, "Standard Tests to Characterize Alfalfa Cultivars", 28 additional check cultivars are now needed. It is the responsibility of this committee to locate seed supplies and arrange to have 15-20 lb. of each cultivar sent to Dr. Austin Campbell at Beltsville for distribution to those making requests. The status of seed supplies and persons contacted to obtain seed are detailed in the table that accompanies this report. At this time, seed of 14 check cultivars has been sent to Dr. Campbell and that of three other checks can be obtained from researchers as specified in the test protocols. Seed increases have been arranged for the other cultivars, as sufficient supplies are not available.

The need for standard tests to describe alfalfa cultivars and differentiate among them is great at present and seems likely to increase in the future. The tests themselves reflect the 'state of the art' in terms of our ability to determine levels of pest resistance and other traits of alfalfa. The current version of the standard tests bulletin allows easy replacement of pages when revised test procedures are distributed and new tests are developed. The committee believes that test protocols will continue to 'evolve' as procedures are improved, new check cultivars become available, and new strains of pests are discovered. Several suggested revisions of the latest protocols have been made by the Alfalfa Variety Review Board. These have been passed on to authors for their consideration.

Currently, two new test protocols are being distributed for review by NAAIC members. These tests have been developed by a special committee chaired by Craig Sheaffer with members including M. Peterson, M. McCaslin, J. Volenec, J. Cherney, K. Johnson, W. Woodward, and D. Viands. One of these is intended to characterize alfalfas with multifoliolate leaves. A point of contention regarding this protocol is whether or not valid ratings can be obtained by visual estimation of multifoliolate leaves per stem. Some members of the special committee believe that visual ratings are adequate, others believe that stems must be clipped and all trifoliolate and multifoliolate leaves counted for valid characterization of this trait. Both procedures are included in the proposed test protocol. The second protocol relates to alfalfa quality factors such as crude protein and fiber content. It has the unanimous support of the committee. These tests will be printed and distributed as soon as appropriate data are assembled on expected levels of expression and acceptable ranges for check cultivars. These data should be available within the next year.

Respectfully submitted:

Richard Berberet, Chair
Cheryl Fox
Craig Grau
Fred Grey
Dave Jessen
Nicole O'Neill
Mike Peterson

STATUS OF SEED SUPPLIES FOR STANDARD CHECK CULTIVARS

May 1992

Report by Richard Berberet, Standard Test Committee

Austin Campbell (USDA, Beltsville) is continuing to handle distribution of seed of check cultivars whenever requests are made. Our goal is to have all check cultivars available from Austin by the end of 1992.

Check Cultivar	Standard Test	Contact Person	Status
Apollo II	Root Knot Nem.	J. Moutray	Seed supplied to A. Campbell
Armor	Sclerotinia	J. Moutray	Seed supplied to A. Campbell
Arrow	Winter Surviv.	J. Moutray	Seed supplied to A. Campbell
AZ-90NDC-ST	Salt Stress	S. Smith	Seed available from S. Smith
AZ-88NDC	Salt Stress	S. Smith	Seed available from S. Smith
DuPuits	Fall Dormancy	D. Barnes	Seed increase - 1992
Excaliber	Winter Surviv.	M. McCaslin	Seed supplied to A. Campbell
KS208	Downy Mildew	D. Stuteville	Seed increase - 1992
Malone	Salt Tolerance	C. Currier	Seed increase - 1992
Mesilla	Fall Dormancy Winter Surviv.	C. Currier	Seed supplied to A. Campbell
MNGRN-1	Fusarium	D. Barnes	Seed increase - 1992
MNGRN-4	Lesion Nematode	D. Barnes	Seed increase - 1992
MSA-CW3-AN3	Com. Leaf Spot	K. Leath	Seed increase - 1992
MSA-PL-L	Lepto Leaf Spot	K. Leath	Seed increase - 1992
Nev. Syn. XX	Root Knot Nem.	R. Peaden	Seed supplied to A. Campbell
Nev. Syn. YY	Root Knot Nem.	R. Peaden	Seed increase - 1992
Norseman	Win. Sur.-Dorm.	D. Barnes	Seed increase - 1992
Ok08	Spot. Alf. Aph.	J. Caddel	Seed supplied to A. Campbell
Ok51	Blue Alf. Aphid	J. Caddel	Seed supplied to A. Campbell
Oneida VR	Verticillium	D. Viands	Seed supplied to A. Campbell
PA-1	Blue Alf. Aph. Pea Aphid	S. Smith	Seed supplied to A. Campbell but seed increase needed
PL-PhR	Sprg. Blackstem	K. Leath	Seed increase - 1992
Rambler	Salt Tolerance	M. Rumbaugh	Seed increase needed
Travois	Yel Leaf Blotch	A. Boe	Seed to be purchased and supplied to A. Campbell
UC-129	Stag. Leaf Spot	D. Irwin	Seed avail. from D. Irwin
Vernema	Stem Nematode	R. Peaden	Seed supplied to A. Campbell
WAPH-1	Aphanomyces	C. Grau	Seed supplied to A. Campbell
WL 316	Clov. Root Cur.	F. Bedard	Seed supplied to A. Campbell

ASTA ALFALFA CROP IDENTIFICATION COMMITTEE
REPORT TO THE NORTH AMERICAN ALFALFA IMPROVEMENT CONFERENCE
JULY, 1992

Committee Members:

Tim Woodward (Chairman)	Pioneer Hi-Bred International, Inc.
Don Barnes	USDA/ARS, University of Minnesota
Jim Elgin	USDA/ARS/NPS, Beltsville, Maryland
Cheryl Fox	Northrup King
Mark McCaslin	Forage Genetics
Tom McCoy	Montana State University
Jim Moutray	AgriPro Biosciences, Inc.
Mike Peterson	W-L Research, Inc.
Don Viands	Cornell University

Recently, the ASTA Crop Minimum Distance Steering Committee changed its name to the "Variety Identification Steering Committee". Subsequently, the Alfalfa Minimum Distance Committee was changed to the "ASTA Alfalfa Variety Identification Committee".

The NAAIC Executive Committee met on June 14, 1992. Tim Woodward presented a potential problem regarding isolation distances and volunteers in seed production fields and the legal affects on future bioengineered varieties. Based on this issue, as well as others, the Executive Committee formed a new standing committee called the "Alfalfa Regulatory Issues Committee". The new committee will replace the NAAIC Minimum Distance Committee and will deal with issues such as:

1. Minimum distance
2. Derivative names
3. Essentially derived
4. PVP application replacement
5. Isolation distances and seed field volunteer affects on varieties with potential genes.
6. Others

Membership of the committee will parallel the ASTA Alfalfa Identification Subcommittee.

The NAAIC Alfalfa Regulatory Issues Committee met for an hour on June 24, 1992. Pierre Gayroud, a member of ASSINSEL, gave a short update on progress regarding forage species. The members of ASSINSEL dealing with forage species have decided to wait on row crops to determine further action. Based on the lack of movement, it was decided to table the discussion on "essentially derived" until more time could be devoted to the issue. The NAAIC Alfalfa Regulatory Issues Committee/ASTA Alfalfa Identification Committee will plan to meet in Minnesota in conjunction with the ASA meetings in November of 1992 to work on the issue.

It was decided that the issue of essentially derived and derivative names could be related. If a variety is considered an EDV, then it would seem appropriate that a derivative name could be used. There was some agreement that greater than 50% relationship would be a minimum requirement for both essentially derived and the use of derivative names.

It appeared that the lack of consistency of checks for winterhardiness and dormancy between the applications for PVP and NAVRB is the major factor slowing the progress of a newly developed PVP application. Don Barnes, senior author for the fall dormancy section of the "Standard Tests to Characterize Alfalfa Cultivars" agreed to identify alternate checks for standard tests for dormancy and winterhardiness in order for both applications to agree.

Submitted by:

Tim Woodward, Chairman

REPORT OF THE COMMITTEE ON THE USE OF BIOTECHNOLOGY RESEARCH IN ALFALFA IMPROVEMENT

T. J. McCoy, D. C. W. Brown, R. W. Groose, L. B. Johnson, and C. P. Vance

Preamble

The 1992 report was compiled from responses received from inquiries regarding biotechnology research at labs around the world. Although we attempted to contact all labs conducting alfalfa biotechnology research, it is likely that significant omissions have occurred. The 1992 report has added a section this year that summarizes worldwide research efforts on *Medicago-Rhizobium* interactions. This section of the report was compiled by Dr. Carroll Vance. In order to meet length restrictions it was necessary to edit most reports. We apologize for any errors in editing including omissions. Please note that names and addresses of contributors are included at the end of this report. We strongly encourage interested parties to contact the individuals listed. This will assist our building of a worldwide network of scientists interested in applications of biotechnology to alfalfa improvement.

United States (Compiled by Lowell Johnson)

At the USDA/ARS, Agricultural Research Center in Beltsville, Maryland, **G. R. Bauchan** and his colleagues have identified RFLPs which can be used to fingerprint self-incompatible clones of alfalfa. These molecular markers have been used to determine the percent hybrids produced when crossing two self-incompatible clones. **T. A. Campbell** and his colleagues have identified an alfalfa root protein (MW ~ 18.7 kD) that is unique to aluminum tolerant clones under aluminum stress and could be the basis for producing a molecular probe for the tolerance response. Utilizing nuclear magnetic resonance (NMR), spin-spin (T_2) water proton relaxation time was consistently shorter in roots under aluminum stress than in unstressed roots, perhaps due to the effects of aluminum on cell membranes. **N. R. O'Neill** is correlating the production of specific enzymes, toxic metabolites, and plasma membrane proteins with cultivar resistance genes. She has identified specific early recognition signals and is determining in whole plant tissues and tissue culture the biochemical changes associated with induced protection operating in tandem with the characteristic defense response to avirulent species and races of *Colletotrichum*. **J. A. Saunders** has developed a novel technique for transforming plants. He electroporates foreign DNA into developing pollen grains which are placed on the pistil of the plant and allowed to develop normally.

At the University of Wisconsin, scientists in several departments are genetically engineering alfalfa to produce enzymes for dominant genetic markers and potential industrial use in making paper and biodegradable detergent. **E. T. Bingham's** project is using GUS as a dominant genetic trait in model breeding and field experiments requiring regulatory approval. In **T. C. Osborn's** lab, a linkage map of diploid alfalfa has been developed in collaboration with researchers at Montana State University and Oregon State University. The map includes over 100 RFLP and RAPD marker loci. RFLP markers are being used to study inbreeding and heterosis at the diploid and tetraploid levels.

At the University of Georgia, **E. C. Brummer** and **J. H. Bouton** and **G. Kochert** are developing a molecular marker linkage map in diploid alfalfa. The map currently contains over 80 RFLP markers. In addition to RFLP markers, they plan to expand the map with RAPD markers. Molecular markers are also being used to conduct studies on genetic variability in annual *Medicago* species. **M. Dall'Agnol, J. H. Bouton** and **W. A. Parrott** are using cell culture techniques, in addition to whole plant selection, to develop alfalfa germplasms tolerant to acid soil and aluminum-toxic soil conditions.

At the University of Nebraska, **M. B. Dickman** is studying race-cultivar specificity and virulence of *Colletotrichum trifolii*. Fungal transcripts have been isolated that appear to be regulated by alfalfa signal molecules that are both race and cultivar specific. *In-vitro* protein phosphorylation experiments suggest that incompatibility (plant defense responses) may be mediated by specific fungal phosphoproteins. **P. Staswick** is testing the feasibility of increasing alfalfa protein content in vegetative tissues by overexpressing soybean vegetative storage protein (VSP) genes. The function of alfalfa genes related to soybean VSP is being studied also.

At the Samuel Roberts Noble Foundation, Ardmore, Okla., the Molecular Plant Pathology Section directed by **R. A. Dixon** is studying gene expression in alfalfa. **N. Paiva** and **J. Blount** are developing strategies for genetically altering phytoalexin synthesis in alfalfa by manipulation of isoflavone reductase (IFR) genes. Assessment of the role of medicarpin in fungal resistance is being made by analyses of transgenic alfalfa expressing antisense IFR, and alteration of medicarpin stereochemistry is being attempted by isolation of IFR genes from species which accumulate medicarpin. **A. Oommen** and **Paiva** are studying the environmental and developmental expression of the the IFR promoter. **C. A. Maxwell** has purified the chalcone 2'-O-methyltransferase responsible for formation of the potent *nod* gene inducer 4,4'-dihydroxy-2'-methoxy chalcone from alfalfa, has cloned its cDNA, and is studying its regulation during symbiosis and defense. **W. Ni** is studying the transcriptional regulation of the lignin biosynthetic enzyme caffeic acid 0-methyltransferase (COMT) from alfalfa and has produced transgenic plants expressing antisense COMT in an attempt to reduce lignin content. **H. Junghans** is pursuing a study of the developmental and environmental control of individual members of the alfalfa chalcone synthase (CHS) multigene family. **M. Harrison** is studying trans-acting factors for expression of CHS genes in alfalfa, and has recently initiated a program to evaluate gene expression in alfalfa mycorrhizal interactions. **T. Fahrendorf** is characterizing an elicitor-inducible cytochrome P450 from alfalfa. **B. Shorrosh** is studying endomembrane proteins from alfalfa and has cloned the protein disulfide isomerase and a homolog of the mammalian glucose regulated protein ERp72. **Shorrosh** is also characterizing isocitrate dehydrogenase and acetyl CoA carboxylase genes from alfalfa. **J. Orr** is studying levels of cinnamic acid derivatives in alfalfa cell cultures in relation to transcriptional regulation of the phenylpropanoid pathway.

Research on organelle inheritance in alfalfa proceeds at the University of Arizona. **N. Forsthoefel**, working with **S. E. Smith** and **H. Bohnert**, has shown that mitochondrial DNA is inherited in a uniparental-maternal fashion in a group of genotypes that exhibit significant variation in plastid inheritance patterns. Using three-dimensional reconstruction of egg cells, **H. L. Mogensen** at Northern Arizona University has also observed that the position of plastids within the egg cell is closely correlated with plastid transmission as a female parent in sexual crosses.

At Northrup-King the field trial conducted in 1989-91 by **C. C. Fox** showed that Basta herbicide resistance in transgenic alfalfa is effective in the field. Field trials with agronomically adapted experimentals resistant to Basta herbicide are planned for 1992.

At the University of Wyoming, **R. W. Groose** is developing diploid *Medicago falcata* genotypes for transformation and cell culture research. A germplasm survey identified two accessions with a high percentage of regenerable genotypes and regeneration was improved via two cycles of recurrent selection. Cultivated alfalfa genotypes that exhibit extensive proliferation of excised roots in vitro are being used to develop a system for monoxenic culture of the root knot nematode. Cell selection to develop selenium-tolerant alfalfa is proceeding also.

At Kansas State University, a rapid method of isolating organellar DNA from alfalfa was developed by **W. Q. Li, D. Z. Skinner, Q. Q. He, T. H. Thin,** and **G. H. Liang. Li** found consistent mtDNA RFLP differences between male sterile and maintainer lines. **Q. Q. He** found RFLP changes in mt and cpDNAs after prolonged growth as callus tissue. **G. Hu** and **D. Z. Skinner** are continuing attempts to transform alfalfa via the pollen tube pathway using a GUS reporter gene and are studying the inheritance of male sterility as related to mtDNA markers. **Skinner** and **D. L. Stuteville** are working to associate RAPD fragments with disease resistance. **C. Chaisrisook, Skinner,** and **Stuteville** are using RAPD fragments in a phylogenetic analysis of five Stemphylium species isolated from alfalfa, and are using pulsed field gel electrophoresis to compare electrophoretic karyotypes of these species. **Skinner** and **D. W. Goad** are refining a method of identifying chromosomal locations of single copy DNA sequences. **X. Ding, L. B. Johnson, F. F. White** and **G. R. Reeck** are using *Agrobacterium tunefaciens* to transform alfalfa with proteinase inhibitor genes in an effort to enhance pest resistance.

At New Mexico State University, **G. C. Phillips, G. D. Kuehn, S. Bagga** and **C. G. Currier** continue to study the role of polyamine biosynthesis and action in relation to drought stress tolerance in alfalfa cell cultures and plants. Isolation of genes corresponding to amino-propyltransferase, polyamine oxidase, and schiff-base reductase are in

progress. **J. A. Henning** and **G. C. Phillips** are developing protocol for asymmetric somatic hybridization technology in *Medicago* spp. **S. Bagga, C. Sengupta-Gopalan** and **J. Kemp** successfully used *Agrobacterium*-mediated transformation to introduce nitrogen fixation by 1) attempting to improve ammonia assimilation through manipulation of the expression of glutamine synthetase genes in roots and nodules and 2) addressing the role of phenylpropanoid-derived compounds on nodule initiation through manipulation of the expression of genes encoding key enzymes in the phenylpropanoid pathway.

At Washington State University, **C. A. Ryan's** group is studying the role of proteinase inhibitors in plant defense. They are transforming alfalfa with foreign proteinase inhibitor genes to study their expression and possible defensive properties against alfalfa insect predators and pathogens. They are also studying chemicals that induce alfalfa proteinase inhibitors to increase defense against predators.

At the University of Minnesota, **D. A. Samac** is investigating defense gene expression in alfalfa and *Medicago* sp. with resistance to the root lesion nematode *Pratylenchus penetrans* and the foliar pathogen *Phoma medicaginis*. She is also developing a PCR-based method for identification of *Pratylenchus* species. Alfalfa germplasm is being evaluated for transformation and regeneration capacity and an *Agrobacterium*-mediated transformation system for alfalfa is being established in the lab.

At the University of Missouri-Kansas City, **J. H. Waterborg** is examining histone variants, in particular histone H3, and their acetylation, in alfalfa tissue culture cells. Cell cycle analysis of transcription and protein synthesis of replication-dependent and constitutive histone H3 variants and their deposition into defined chromatin domains is in progress.

At the University of Nevada-Reno, **I. Winicov** is studying alfalfa genes involved in salt tolerance in tissue culture cells and in whole plants. Salt tolerant plants have been regenerated from salt tolerant cell lines and the tolerance trait is transmissible and appears to be partially dominant in the selfed progeny. Several cDNA clones have been isolated from the salt tolerant cell lines that represent genes activated by salt.

At Pioneer Hi-Bred International, the breeding program directed by **W. T. W. Woodward** has successfully moved the AMV coat protein gene into four dormant and one nondormant experimentals through the use of modified backcrosses. Kanamycin was used as a selection marker with ELISA and PCR technology used to confirm selected progeny containing the gene during the breeding process. Field tests will be planted in Washington, California, Iowa, Wisconsin, and Pennsylvania to study the effects of the AMV coat protein gene. Entries include the five experimentals, two pairs of isogenic lines for the AMV coat protein gene and recognized check varieties. Both the particle gun and *Agrobacterium*-mediated transformation work are continuing. **K. Dalkin** will head the alfalfa transformation lab.

At Montana State University, **T. J. McCoy** and **C. S. Echt** have developed a 100+ RFLP and RAPD marker linkage map of diploid alfalfa in collaboration with researchers at the University of Wisconsin and Oregon State University. Molecular marker loci-centromere map distances currently are being determined by the use of first division restitution 2n pollen in 4x-2x crosses. Analysis of segregation and recombination of molecular markers in progeny of interspecific hybrids is being used to determine genomic affinity, in conjunction with cytogenetic analysis. In addition, transformation research has focused on analysis of transgenic plants and progeny carrying an insect proteinase inhibitor (PI) gene from the tobacco hornworm (*Manduca sexta*). Expression studies have demonstrated that various parameters affect expression of this PI gene in transgeneic alfalfa including promoter, type of tissue, and growth stage of the plant.

Australia, Japan and People's Republic of China
(Compiled by Tom McCoy)

At CSIRO Division of Plant Industry in Canberra, Australia, alfalfa biotechnology research is focused on the following: Improving transformation protocols for commercial Australian winter-active cultivars (**H. Schroeder, T. Wardley-Richardson**); Cloning genes for condensed tannin (proanthocyanin) synthesis to be transferred to alfalfa to produce bloat-safety and improved by-pass protein for grazing ruminants-- also asymmetric somatic hybridization with the same objective (**G. Tanner, F. Paolocci, Li Yuguang, P. Larkin**); Somatic hybridization between alfalfa and *Lotus spp.* to transfer acid soil tolerance to alfalfa (**P. Stoutjesdijk, P. Larkin**); Cloning genes from the lignin synthesis pathway and combining these with stem-specific ribozyme constructs in order to reduce lignification and improve the quality of alfalfa stems (**S. Abrahams, R. Sandeman, J. Watson**); Improving the sulfur-amino acid nutritional status of alfalfa forage protein by genetic engineering using various genes encoding proteins with a high content of sulfur-amino acids (**L. Tabe, W. McNabb, R. Morton, C. Higgins, T. J. Higgins**); Alfalfa mosaic virus resistance using coat protein genes (**P. Chu, T. J. Higgins**).

At the National Grassland Research Institute in Tochigi, Japan, **K. Suginobu** is conducting research on the following: Efficient induction of somatic embryos of alfalfa for artificial seed development; *In vitro* cell selection for aluminum tolerance in alfalfa; Development of acid soil tolerant alfalfa by cell fusion with Japanese native legumes such as *Indigofera pseudo-tinctoria Matsum.* Also at the National Grassland Research Institute, **K. Okumura** and **M. Kannbe** are applying tissue culture systems to the multiplication and conservation of alfalfa breeding materials.

At the Institute of Animal Science, Chinese Academy of Agricultural Sciences in Beijing, PRC, **Li Cong** has selected salt-tolerant cell lines of alfalfa. Compared to the original cultivar, average yield and survival of progeny plants by cell selection was raised 14 percent and 18 percent, respectively, under 0.3 percent NaCl stress. In cooperation with **Chen Shou Yi's** group (Genetics Institute, Academia Sinicia, Beijing) they are focusing on sequence analysis, isolation and cloning of genes controlling osmotic regulation.

Africa and Europe (Compiled by R. W. Groose)

In Kostinbrod, Bulgaria, at the Institute of Genetic Engineering, **A. Atanassov** and **P. I. Denchev** are studying development of somatic embryos; **E. Marinova** obtained alfalfa plants resistant to PEG and NaCl; and **M. Vlahova** developed methods for gene transfer using both *Agrobacterium tumefaciens* and *A. rhizogenes* vectors.

In Czechoslovakia, **E. Krajci** initiated a new program with alfalfa at the Research and Breeding Institute in Bucany. Current work involves micropropagation of breeding materials using shoot-tip cultures and an attempt to produce haploids using pollen and ovule cultures.

In Italy, at the Institute de Ricerche sul Miglioramento Genetico delle Plante Foraggere del CNR at Perugia, the laboratory of **S. Arcioni** is studying the transfer of traits from non-cultivated *Medicago* species into alfalfa via protoplast fusion. They are also attempting to transfer genes for condensed leaf tannins into alfalfa from *Onobrychis viciifolia* using asymmetric somatic hybridization, investigating *Agrobacterium*-mediated transformation for introducing maize genes for sulphur-rich proteins into alfalfa, using embryo rescue for producing hybrids between *M. sativa* and wild (annual and perennial) *Medicago* species, and investigating RFLP technology for genetic analysis of somatic hybrids, cultivar distinctiveness and agronomic traits.

In South Africa, at the Institute for Plant Biotechnology in Pretoria, **A. L. Kotze** and colleagues have initiated a program to improve alfalfa by transformation with cloned genes involved in pathogen resistance. They are investigating altered chalcone synthase, phenylalanine ammonia-lyase and chitinase gene expression during *Colletotrichum trifolii*-infection. They are also investigating RAPD markers for cultivar identification.

Canada and Mexico (Compiled by D. C. W. Brown)

At the National Polytechnic Institute in Mexico, **T. Villegas** and her group are attempting to increase the accumulation of storage proteins within the somatic embryos of alfalfa. They are also studying the correlation between the levels of storage proteins and the quality of somatic embryos, measured in terms of ability to regenerate plants.

At Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. (CIATEJ, A.C.) in Guadalajara, México, **B. Rodriguez-Garay** and his group are working with cell selection for salt tolerance in alfalfa. They have screened about 600 genotypes of the Mexican variety, Atoyac, for regenerative ability and only one out of those 600 genotypes was found to be embryogenic.

The Flavonoid Biotechnology program is comprised of **B. Goplen, M. Gruber, C. Hinks, G. Lees, A. Muir** and **S. Wright** at the Saskatoon Research Station of Agriculture, **K. J. Cheng**, at the Lethbridge Research Station and **B. Bohm** and **N. Towers** in the Dept. of Botany at the University of British Columbia. The group is studying the expression of monomeric flavonoids/condensed tannins and their enzymes as a function of plant development with a view to genetically engineer alfalfa to express condensed tannins in leaves. The composition and content of a number of condensed tannin molecular species which are differentially accumulated during leaf and seedcoat development have been partially characterized and the cellular and subcellular localization of these compounds has been analyzed. An antisense flavonoid gene has been used to transform sainfoin leaves, and some of the plantlets do not produce condensed tannins. Grasshoppers appear to be severely affected by the presence of condensed tannins in plants if the plant has a low protein content.

At the University of Guelph, **S. R. Bowley** and colleagues are breeding populations of alfalfa for use in commercial utilization of somatic seed technology. **S. R. Bowley** and **B. D. McKersie** are evaluating field and laboratory stress tolerance of transformed alfalfa which are expressing additional superoxide dismutase and alcohol dehydrogenase genes. **B. D. McKersie, T. Seneratna** and colleagues are developing the artificial seed technology to improve somatic embryogenesis, embryos maturation, and desiccation. **L. Erickson** and colleagues are using genomic, cDNA, and PCR cloning to isolate DNA sequences in alfalfa which affect pollen development and which, in conjunction with particle bombardment, will be used to engineer male sterility and enhance vector integration. **K. P. Pauls** has developed procedures, based on RAPD technology, for estimating genetic relatedness among tetraploid alfalfa populations.

In the Botany Department at the University of Guelph, **J. D. Bewley** and coworkers **J. E. Krochko, S. K. Pramanik** and **N. Xu** are studying the regulation of storage protein synthesis in developing zygotic and somatic embryos. They have obtained antibodies and cDNA clones to the major 11S, 7S and 2S storage proteins embryos. Failure of synthesis during early development is due to trapping of transcripts in an mRNP fraction. A new technique has been developed for rapid and efficient screening of a cDNA library without the requirement for using bacterial colonies; numerous alfalfa clones have been obtained in this way.

At McGill University **R. S. Dhindsa** and co-workers have cloned and sequenced several cDNAs corresponding to genes, the expression of which occurs specifically during cold acclimation and is correlated positively with cultivar freezing tolerance. They are also studying the molecular biology of low temperature signal transduction in alfalfa.

At the Sainte-Foy Research Station of Agriculture Canada, **Y. Castonguay, S. Laberge, L-P. Vezina,** and **R. Michaud** are developing an *Agrobacterium*-based transformation system for alfalfa to evaluate the role of isolated genes cold acclimation. Three genes associated with the cold acclimation process have been isolated and are being evaluated with respect to their role during cold acclimation.

J. Nowak and **S. L. Matheson**, Nova Scotia Agricultural College, have selected variants (from callus and cell suspension cultures) and genotypes (from seedlings) resistant to the proline analogues, trans-4-hydroxy-L-proline and L-azetidine-2-carboxylic acid, to upgrade cold stress tolerance of locally adapted alfalfa cultivars. The selected germplasm is presently being evaluated for freezing tolerance.

At the Plant Research Centre, Agriculture Canada, **L. Barran, E. S. P. Bromfield, D. C. W. Brown and A. R. McElroy** are collaborating on wide hybridization studies between *M. sativa* and *M. lacinata* and *M. truncatula* to modify the host range specificity and in vitro regeneration characteristics of alfalfa. **E. S. P. Bromfield** is working on the impact of legume genotype on the composition of natural populations of *Rhizobium meliloti* and on the establishment of inoculant strains in the crop ecosystem. Data from the studies with various *Medicago* and *Melilotus* species suggest that the legume host is a major factor influencing the composition of its associated *Rhizobium* population. **A. R. McElroy** is studying the in vitro regeneration characteristics of alfalfa during the transfer of the genes responsible for somatic embryogenesis into elite breeding material. He is also developing a transplant plug system for delivery of clonally propagated material, suitable for hybrid alfalfa parental lines, into the field and evaluating transformed, herbicide resistant alfalfa performance in the field. **D. C. W. Brown, E. Svanbaev** and colleagues are developing the artificial seed technology by focusing on improving embryo quality, studying gene expression during in vitro embryo development and working on the production of somatic embryos in bioreactors. They have also developed a transient expression system based on the particle bombardment method and are working on developing a stable gene expression method based on the particle bombardment transformation approach.

The Legume Biotechnology group of the Plant Biotechnology Institute of the National Research Council, headed by **J. Mahon,** has added alfalfa to its list of actively-studied legumes. **L. M. Nelson** has added cell suspension cultures of alfalfa to those of tobacco and pea for studying the effect of culture conditions on *Agrobacterium* vir-region induction and the transformation of plant cells. **L. Gedamu** at the University of Calgary and **S. Misra** at the University of Victoria are investigating the transformation and regulated expression of metalothione genes in alfalfa for use in the remediation of contaminated soils.

Alfalfa-*Rhizobium* Interactions (Compiled by C. P. Vance

At the University of Tennessee, **G. Caetano-Anollés** is studying mechanisms that regulate the initiation and development of nodules, spontaneous nodulation in the absence of *Rhizobium* plant determinants of host-specificity.

D. Barker, J. Cullimore, E. Journet, T. Huguet and D. Jean at INRA-CNRS in France are using the diploid autogamous *Medicago truncatula* as a model system to study the nitrogen-fixing symbiotic interaction between *Medicago* and *Rhizobium*, combining both molecular and genetic approaches. *M. truncatula* genes have been isolated which are implicated in both the early steps of infection and nodule development, and in the later nitrogen-fixing stages of development. The characterization of nodulation-defective mutants of *M. truncatula*, is also an important research priority, and the detailed cytological and molecular analysis of a novel Fix$^-$ mutant is currently underway.

In Rome, Italy, **R. DiBonito** is selecting *Rhizobium meliloti* strains with good competitive ability for nodulation on alfalfa in Italian soils. A major focus of this research is determining the antagonism between rhizosphere microorganisms.

P. J. Bottomley at Oregon State University is studying the influence of indigenous soil rhizobial populations upon yield, N_2-fixing potential and cultivar characteristics under field conditions.

Research interests of **R. Dickstein** at Drexel University include plant genes expressed in nodules, especially those expressed empty nodules, and development of *Medicago truncatula* as a model legume.

G. Duc and M. Sagan at INRA-Dijo-Cedex, France, are searching for mutants affecting nodulation and nitrogen fixation in *Medicago truncatula.*

At the Pennsylvania State, Berks Campus in Reading, **B. D. Eardly** is studying molecular population genetics of *Rhizobium.* He is examining the extent and range of genetic variation in *Rhizobium* at the level of structural genes.

The subjects he is using are *R. meliloti* strains from geographically diverse regions, including southwest Asia, which appear to be a center for genetic diversity in the *Medicago*. His collection currently includes over 200 strains of *R. meliloti* that have been characterized for chromosomal genetic variation.

In Hungary, **T. Gyori** is conducting cloning efficiency studies using auxin hormones. He is studying the role of genetic variability in cloning of alfalfa cultivars.

G. Hérnandez in Cuernavaca, Mexico, is studying regulation of control processes for the *Rhizobium meliloti*-alfalfa symbiosis. Her approach is to modify the expression of genes that code for key enzymes of the symbiotic process and to study the physiological effect of these modifications.

D. Helinski and G. Ditta at the University of California-San Diego, are interested in the molecular control of nitrogenase expression in *Rhizobium meliloti* and the genetic regulation of nif genes.

A. Hirsch's group at the University of California-Los Angeles, is examining nodule development and early nodulin gene expression in alfalfa. They have isolated a number of cDNA clones for proline-rich proteins from an alfalfa cDNA library. They are also isolating cDNAs and genes for enzymes of the phenylpropanoid pathway. *In situ* hybridization techniques to localize transcripts of various nodulin genes within nodule tissues is the approach being used.

At Washington State University, **M. L. Kahn** is studying nitrogen and carbon assimilation during root nodule symbiosis and nutrient exchange between host plant and nodule bacteroides.

Dr. G. B. Kiss and his colleagues in Szeged, Hungary, have isolated and characterized two nodule specific genes, leghemoglobin and Nms-25 (nodulin-25-from alfalfa. They have isolated three mutant plants, Fix-1, Fix-2 and Fix-7 from diploid *Medicago coerulea*. The aim of this work is to map Fix-alleles and eventually clone them.

A. Kondorosi at the Institut des Sciences Végétales, CNRS, Gif-sur-Yvette Cedex, France, is working on the molecular genetics of *R. meliloti-Medicago* interactions. His lab is studying recognition and transduction of Nod signals and identifying plant genes expressed early after *Rhizobium* infection.

S. Long at Stanford University, Stanford, Calif., is studying the molecular control of root nodule initiation and development. Bacterial genes affecting nodulation and signaling between microbes and plants are being analyzed.

F. O'Gara in Cork, Ireland, is examining the molecular biology of gene expression in *R. meliloti*, genetic regulation of carbon metabolism (dct, cya, crp genes) and marker genes and stable vectors for deliberate release of inoculants.

Research interests of **D. A. Phillips** at the University of California-Davis, includes alfalfa rhizosphere ecology, root biology, and soil microbiology.

The laboratory of **C. P. Vance** at the University of Minnesota, St. Paul, Minn., focuses on the biochemical, physiological, and molecular features that are involved in root nodule function and nitrogen assimilation. They also are interested in factors which mediate compatibility between the host plant and microbe.

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NATIONAL ALFALFA VARIETY REVIEW BOARD REPORT

Joe Bouton, Cliff Currier, and Craig Grau
University of Georgia, New Mexico State University,
and University of Wisconsin, Respectively

1991

The annual meeting of the National Alfalfa Review Board (NAVRB) was held on 9-11 January 1991 at the Campri Hotel in Boise, Idaho where the board reviewed 58 applications. Several items were discussed at the business meeting including the following main points:

1. Motion passed that the Board recommend to the Certified Alfalfa Seed Council (CASC) that only alfalfa alfalfa varieties passed by the NAVRB and accepted by PVP be included in the Alfalfa Variety publication.

2. Motion passed that the alternate ASTA-Research representative on the Board be appointed to compile data for the Alfalfa Variety publication and forward it to the CASC.

3. Suggestion made that the idea of a permanent secretary for the board be brought before the AOSCA Executive Committee for their reaction.

4. Request made by the board to all applicants that if a name is given to a variety after Board action, that the developer contact the AOSCA office or the Chair, NAVRB.

5. Motion passed to draft a letter to Pioneer with copies to all industry personnel asking that they change their wording in advertisements claiming NAVRB approval of a "winter hardiness" claim.

6. Motion passed that minutes reflect that the NAVRB knows of no standardized test for cold tolerance or winterhardiness.

In the interim between the 1991 and 1992 meetings, the following business was conducted by mail: 1) changes were made in the NAVRB application form and 2) a letter was sent by the chairman on September 6, 1991 to all NAVRB members and potential variety applicants announcing that the board would review all applications in line with the new edition of the "Standard Tests to Characterize Alfalfa Cultivars" manual with the board accepting check varieties from either the old or new bulletin and where new resistance ranges have been established for an old check variety, only these new ranges will be used.

1992

The annual meeting of the NAVRB was held on 6-8 January at the Airport Hilton in Kansas City, MO. The board reviewed 61 applications and the following main points were made either

during the discussion before the review process or at the business meeting:

1. The board agreed to the following when reviewing the current applications in keeping with the letter sent on September 6:
    a. If a resistant or susceptible check does not appear in either the old or new book of standard tests, the test will be thrown out.
    b. If the checks do no fall within the prescribed ranges per the procedures described in either publication on an unadjusted basis, the test will be thrown out.
    c. The board will recognize data from either space plants or plots for fall dormancy, but beginning next year will require data from space plants only with checks as outlined in the new manual.

2. Discussed the draft copy of the "Standard Test to Characterize Alfalfa Cultivar Forage Quality". NAAIC will continue to work on this procedure.

3. Discussed "Comparison of Multilef Expression" report. Work will continue by NAAIC on this report.

4. Heard a strong plea from those in attendance that changes in board policy must be announced at least 10 months before applications are due and not as was done with the September 6 letter.

5. Made changes in the NAVRB application form.

In February 1992, the board, by mail ballot, decided to have the chairman rereview the applications rejected due to the changes made in the September 6 letter and bring all rejected applications and tests more in line with what was board policy in January 1991. This was done by the chairman and the incoming chairman. However, the board wishes to announce that the new application form and the 1991 "Standard Tests to Characterize Alfalfa Cultivars" will be used for all applications reviewed during the upcoming 1993 meeting to be held in Atlanta, GA on 4-6 January.

AWARDS COMMITTEE REPORT

Nominations were solicited for the NAAIC, Honorary Membership Award and the Richard R. Hill, Jr. Achievement Award to be awarded at the 33rd NAAIC. Five awardees for the Honorary Membership were chosen by the committee, as well as a recipient for the Richard R. Hill, Jr. Achievement Award. Nominees not chosen for the Honorary Membership at the 33rd NAAIC will automatically be reconsidered for the 34th NAAIC in 1994.

Members of the Awards Committee included Donald Brown, Donald Graffis, John Kugler, Real Michaud, Craig Sheaffer, and Kenneth Leath (Chairman).

1992 NAAIC HONORARY MEMBERS

DONALD C. ERWIN: A native Nebraskan, Dr. Donald C. Erwin received his doctorate from the University of California at Davis. His dissertation research characterized the involvement of Stagonospora meliloti and Rhizctonia solani in crown rot of alfalfa. Dr. Erwin was the first to prove that a root disease of alfalfa, later to be shown to be of worldwide importance, was caused by Phytophthora megasperma f.sp. medicaginis. Dr. Erwin and his co-workers developed four cultivars or germplasm lines of alfalfa with resistance to Phytophthora that have been of great benefit to the industry. Dr. Erwin published a series of papers on factors influencing sporulation of Phytophthora, inoculum dissemination, and infection of the plant. In a 1967 paper in Nature, Dr. Erwin reported genetic recombination in germinated oospores of P. infestans which was followed by a paper describing variation in pathogenicity. These efforts culminated in a paper, co-authored with Dr. George Zentmyer, on development and reproduction in Phytophthora. His publications concerned with Phytophthora cover a 37-year period through 1990 clearly confirming his long-term commitment to the study of Phytophthora and chronicles his many contributions to the improvement of alfalfa and its management. Dr. Erwin was the first to demonstrate that xylem necrosis and death of alfalfa following flood irrigation during the summer was due to the interaction of high soil moisture content and high temperature. He also continued his early interest in Stagonospora with the development of screening methods for genetic resistance and the release, with W. F. Lehman, of UC 129, the first cultivar with moderate resistance to Stagonospora leaf spot and crown rot. More recently, Dr. Erwin has studied the introduction and distribution of the alfalfa infecting strain of Verticilium albo-atrum in California and has collaboratively developed several lines of alfalfa resistant to Verticillium wilt, which are now in the final stages of testing.

BERNARD P. GOPLEN: Dr. Bernie P. Goplen is a native of Saskatchewan, Canada who received his graduate training at the University of California at Davis. He is an internationally recognized expert on legumes in the northern Great plains, and has authored 70 scientific publications and over 130 reports and technical publications. His investigations have covered many facets of alfalfa research, including management of forage and seed production, diseases, the role of leafcutteer bees in seed production, cold tolerance, genetics and cytology. Dr. Goplen has played a key role on the development of research programs on forage legumes for the northern prairies, and has had a major impact on the development and implementation of quality standards for forage seed in Canada. In his employment with Agriculture Canada Research Branch, Dr. Goplen has devoted his professional career to the improvement and utilization of legumes on the prairies. To this end he had developed and registered seven legume cultivars, of which two are alfalfas. The cultivars produced by Dr. Goplen dominate the Canadian prairie market. He currently heads a multidisciplinary team whose aim since 1972 has been the development of a non-bloating alfalfa through selection of a slower initial rate of digestion character, a project which is now nearing completion. More recently, he has been leading a program which will use molecular biology and genetic engineering techniques to transfer condensed tannins, known non-bloating traits, from other legumes to alfalfa.

DARRELL A. MILLER: Dr. Darrell A. Miller has pioneered in alfalfa breeding and genetics, and in the education of students in the principles of alfalfa breeding, genetics, production, and utilization. His research in alfalfa breeding and genetics has led to the release of 8 germplasms that were used in at least 27 cultivars in the United States. Over the years, nine of these cultivars have ranked among the top 10 for the state of Illinois. The germplasm releases provided the alfalfa industry with breeding material having heritability for high protein, weevil tolerance, high yield, and branched rooting. His pioneering research into the mechanisms of allelopathy and autotoxicity in alfalfa has provided explanations for failure of stand establishment and low productivity, and has identified secondary metabolites of alfalfa that contribute to the undesirable allelopathic and autotoxic problems. His pioneering research on the branched rooting character has opened opportunities for midwestern producers to reestablish alfalfa on poorly drained, shallow soil. Dr. Miller has been instrumental in developing and promoting the alfalfa cubing industry in Illinois, and in promoting the Total Mixed Ration concept with the alfalfa industry. Through his popular 1984 textbook FORAGE CROPS, and his co-editorship of the forthcoming revision of the premier textbook FORAGES: THE SCIENCE OF GRASSLAND AGRICULTURE, Dr. Miller assures that sound principles of alfalfa science, production, and utilization will benefit the future public and private sector leaders of the alfalfa industry.

DONALD K. BARNES: Dr. Donald K. Barnes is a product of Minneapolis and earned all his degrees at the University of Minnesota. Dr. Barnes has been involved in alfalfa research for more than 36 years, including 30 years as a research geneticist with USDA-ARS. He has made significant research contributions in many areas including the development and improvement of alfalfa germplasm; in development of methodology for identifying disease, nematode, and insect resistant alfalfa germplasm; and breeding for improved nitrogen fixation. He has also been active in technology transfer activities such as co-editorship of the text ALFALFA AND ALFALFA IMPROVEMENT. He has served in many leadership capacities such as 10 years as permanent secretary (now executive secretary) of the North American Alfalfa Improvement Conference and chairman of the Alfalfa Crop Advisory Committee. In addition, Dr. Barnes has trained more than 25 undergraduate and 30 graduate students, many who are now making significant contributions in alfalfa breeding. The disease nurseries maintained at Minnesota by Dr. Barnes have for many years provided the alfalfa industry with a unique and extremely valuable evaluation of their breeding lines.

JOHN E. BAYLOR: Dr. John E. Baylor was born in New Jersey and received his bachelor's and master's degrees from Rutgers University and his Ph.D. from Penn State University. Dr. Baylor is Professor Emeritus of Agronomy at Pennsylvania State University. He retired in 1983 and at that time joined Beachley-Hardy Seed Company as Director of Market Development and now acts as a consultant to the industry. In 1987 he assumed additional responsibility as Executive Director of the Atlantic Seedmen's Association. As Extension Forage Specialist at Penn State for 26 years, he conducted many innovative programs including the Alfalfa Growers Program and in-depth Forage Workshops for producers and agri-industry personnel. His educational programs received national attention. He has been an active member of the ASA for over 40 years and chaired a number of the Society's committees. He was a charter member of the Pennsylvania Forage and Grassland Council, served as the council's first president and then for 25 years served as its Executive Vice President. He has been associated with the Certified Alfalfa Seed Council since it was formed and served on its advisory board for approximately 25 years. He has also been a long time member of the American Forage and Grassland Council having served on its Board for nearly 20 years and as President. Internationally, John was active in the organization of the IX International Grassland Congress in Brazil and was Chairman of the Governing Board for the XIV International Grassland Congress held at the University of Kentucky. He has received a number of awards including the AFGC Medallion Award and the Certified Alfalfa Seed Council's Industry Award. John Baylor's service to alfalfa had touched every aspect of the industry.

R. R. HILL AWARD

BARBARA W. PENNYPACKER: Dr. Barbara White Pennypacker is a native of Pennsylvania and received her undergraduate and graduate degrees from The Pennsylvania State University. Dr. Pennypacker worked, on a part-time basis, as a Research Associate in the Department of Plant Pathology at Penn State for about fifteen years while raising a daughter. Twenty years after receiving her master's degree, she received her Ph.D. degree with a dissertation on Verticillium wilt of alfalfa. Dr. Pennypacker, as a part-time researcher and graduate student, developed an in-depth and multifaceted investigation into this disease, and her ten publications constitute the main core of our knowledge on this disease. Her findings include the pathogenic mechanisms of how this pathogen invades, spreads and disrupts normal water conducting processes in susceptible plants, as well as the behavior of this pathogen and its effects in resistant plants. Of particular interest is the capability of the pathogen to disrupt photosynthesis in susceptible but not in resistant plants. Also, Dr. Pennypacker observed the formation of lignitubers as a defense response in resistant plants. This is the first report of their occurrence in xylem vessels. Another accomplishment of Dr. Pennypacker was a system to induce gradual drought stress, which she used to demonstrate the stability of resistance to Verticillium wilt when alfalfa was under drought stress. Honors received by Dr. Pennypacker include domestic and foreign invitational presentations, the Outstanding Graduate Student Research Award in Science by Penn State University, a Research Grant from the Richard Storkan Foundation, and a USDA Competitive Grant to continue her research into the role of sustained photosynthesis as a characteristic of wilt-resistant plants.

1992 NAAIC Award Winners (L-R) - J. E. Baylor, B. P. Goplen, B. W. Pennypacker, D. K. Barnes, and D. A. Miller. (D. C. Erwin, not available for picture.)

PREVIOUS NAAIC HONORARY MEMBER AWARDEES

Conference	Awardees	Date
30th NAAIC	David F. Beard C. S. (Gary) Garrison David H. Heinrichs Hewitt M. Tysdal	July 30, 1986
31st NAAIC	Clarence H. Hanson Royse P. Murphy Dale Smith	June 23, 1988
32nd NAAIC	Robert R. Kalton William R. Kehr Vern L. Marble Edgar L. Sorensen	August 23, 1990

RESOLUTIONS COMMITTEE REPORT

Be it resolved that the joint meeting of the 33rd North American Alfalfa Improvement Conference and the 27th Forage Insect Research Conference, in session at Atlanta, Georgia, on June 14-18, 1992, convey their sincere and enthusiastic appreciation to:

The Executive Committee of the NAAIC and the FIRC for conceiving the plans for these outstanding meetings, including, Gary Bauchan, Program Chair, for organizing the meeting program and Jim Moutray, NAAIC President, for his dedication and leadership:

The Local Arrangements Committee from the University of Georgia: Joe Bouton (Chair), Nick Hill, Al Smith, Carl Hoveland, Roger Gates, and Stan Wilkinson; to their students and technicians -- Charlie Brummer, Miguel Dall'Agnol, Donald Wood, and Greg Durham, who did much of the detail work;

The University of Georgia, Agronomy Department, for providing staff, facilities, and warm hospitality;

Vaughn Calvert, Frank Newsom, and the entire field staff at the Univ. of Georgia's Central Georgia Branch Station for the tour of the station;

Wayne Tunkersly (County Ext. Spec., Madison), Mark McCann (UGA Anim. Sci.) and the Little River Farm staff for their insight into alfalfa production in Georgia;

Linda Montgomery, Cathy Biel, and the entire Holiday Inn Staff for their hospitality and service;

Joe Bouton for serving as Master of Ceremonies of the Awards Banquet and to Bobby Rowan for his humorous and interesting presentation at the banquet;

Gary Payne and the staff at the USDA Fruit and Nut Station at Byron for the station tour;

Roger Gates, Glenn Burton, Wayne Hanna, Jeff Wilson, Max Austin, Phil Utley, and the staff at the Coastal Plains Experiment Station at Tifton for the overview of their activities; and

Stewart Newberry (County Ext. Spec.) and the Musstock Dairy staff for the view of dairying in the south.

We also want to extend a special thanks to the many commercial organizations that generously support the activities of the conference through their sustaining membership contributions.

Appreciation is equally conveyed to the contributing scientists for their excellent oral and poster presentations communicating the latest in alfalfa science, ie., alfalfa improvement and forage insect research; to the professional participants in conference activities; and to all the others involved in making this meeting a memorable and informative.

Respectfully Submitted,
John Caddel and Thad Busbice (Chair)

LOCATION COMMITTEE REPORT

The committee assignment was to recommend a site for the 1994 North American Alfalfa Improvement Conference and to suggest sites for subsequent conferences. This was accomplished by phone, letter, fax, and direct conversations during the conference.

An invitation was received to hold the 1994 NAAIC in Guelph, Ontario, Canada. Steve Bowley has extended the invitation for the University of Guelph. He and several colleagues proposed Sunday through Thursday of the last week in June for the meeting. It is recommended that the NAAIC Executive Committee accept this invitation. Dr. Brian McKersie orally added to the invitation. There was a brief discussion about conflicts that week. The Executive Committee will work out dates with the group at Guelph.

In keeping with the last Location Committee Report (1990), we suggest that the 1996 conference be held in Oklahoma, hosted by Oklahoma State University. It is also suggested that Montana State University host the meeting in 1998. Suggestion for subsequent meeting sites should be made to the Location Committee chairman or to the Executive Committee.

Submitted by
John Caddel (Chair) and John Kugler

Nominating Committee Report

The NAAIC Bylaws state that the succession of officers from Secretary to Vice President to President shall alternate between public and private institutions. The Secretary for the 1992-94 conference period is to be from a public institution. Therefore, the Nominating Committee nominates the following individuals as NAAIC officers:

President	G. R. Bauchan	USDA-ARS
Vice President	M. McCaslin	Forage Genetics
Secretary	R. Michaud	Agriculture Canada

All of these nominees have agreed to serve in these positions.

In accordance with the Bylaws, the Nominating Committee consisted of the following individuals:

D. W. Evans	WAIC Chair
W. T. W. Woodward	CAIC Chair
A. R. Gotlieb	EFIC Chair
C. Fox	Industry Committee Chair
B. P. Goplen	Canadian Representative
D. R. Viands (Chm.)	Immediate Past President

History of the North American Alfalfa Improvement Conference

No.	Year	Location	Chairman	Secretary
1	1934	Lincoln, NE	T. A. Kiesselbach	H. M. Tysdal
2	1934	Washington, DC	A. J. Pieters	H. M. Tysdal
3	1935	St. Paul, MN	H. L. Westover	H. L. Westover
4	1936	Madison, WI	R. A. Brink	H. M. Tysdal
5	1937	Chicago, IL	R. A. Brink	H. L. Westover
6	1938	Manhattan, KS	H. M. Tysdal	H. L. Westover
7	1939	New Orleans, LA	H. M. Tysdal	H. L. Westover
8	1940	Ft. Collins, CO	L. F. Graber	H. L. Westover
9	1942	St. Louis, MO	L. F. Graber	H. M. Tysdal
10	1946	Logan, UT	J. W. Carlson	H. M. Tysdal
11	1948	Lincoln, NE	C. O. Grandfield	H. M. Tysdal
12	1950	Lethbridge, Canada	T. M. Stevenson	O. S. Aamodt
13	1952	Raleigh, NC	R. P. Murphy	O. S. Aamodt
14	1954	Davis, CA	O. F. Smith	H. O. Graumann
15	1956	St. Paul, MN	C. P. Wilsie	H. O. Graumann
16	1958	Ithaca, NY	C. H. Hanson	H. O. Graumann
17	1960	Saskatoon, Canada	J. L. Bolton	C. H. Hanson
18	1962	Davis, CA	E. H. Stanford	C. H. Hanson
19	1964	Lafayette, IN	R. L. Davis	C. H. Hanson
20	1966	University Park, PA	H. L. Carnahan	C. H. Hanson
21	1968	Reno, NV	W. R. Kehr	C. H. Hanson
22	1970	Urbana, IL	R. R. Hill, Jr.	C. H. Hanson
23	1972	Ottawa, Canada	D. H. Heinrichs	C. H. Hanson
24	1974	Tucson, AZ	Dale Smith	C. H. Hanson & D. K. Barnes
25	1976	Ithaca, NY	M. S. Pedersen	D. K. Barnes
26	1978	Brookings, SD	M. D. Rumbaugh	D. K. Barnes
27	1980	Madison, WI	E. L. Sorenson	D. K. Barnes
28	1982	Davis, CA	B. A. Melton	D. K. Barnes
29	1984	Lethbridge, Canada	R. R. Kalton	D. K. Barnes
			President	**Executive Secretary**
30	1986	Minneapolis, MN	B. P. Goplen	J. H. Elgin, Jr.
31	1988	Beltsville, MD	W. J. Knipe	J. H. Elgin, Jr.
32	1990	Pasco, WA	D. R. Viands	J. H. Elgin, Jr.
33	1992	Atlanta, GA	J. B. Moutray	J. H. Elgin, Jr.

North American Alfalfa Improvement Conference (NAAIC)
Mailing List Questionnaire

Returning this questionnaire indicates that you would either like to be added to the NAAIC mailing list or that you have an address or activity change.

Date: _____

1. Please check one of the following:

New address _____ New member _____ Activity Change _____

PLEASE TYPE OR PRINT

2. Name: _____

3. Mailing Address: _____

_____

_____

Telephone No.: _____ Fax No.: _____

4. Present activities with alfalfa: Check appropriate blank(s):

Research Activities	Non-Research Activities
A ____ Breeding	K ____ Administration
B ____ Entomology	L ____ Extension
C ____ Pathology	M ____ Forage Producer
D ____ Nematology	N ____ Marketing
E ____ Physiology & Microbiology	O ____ Seed Producer
F ____ Molecular Biology	P ____ Student
G ____ Forage Production	Q ____ Teacher
H ____ Seed Production	R ____ Certification & Variety Protection
I ____ Utilization	
J ____ Chemical & Quality Analysis	S ____ Writer or Publisher

For Canadian and USA Scientists Only:

Which Regional Alfalfa Improvement Conference(s) would you like to receive information about? Eastern ___ Central ___ Western ___

What best describes your employment situation: USDA ___, SAES ___, U.S. Private Industry ___, Canadian Public ___, Canadian Private ___

Would you like new variety and germplasm release information? ___

5. Return To: Dr. James H. Elgin, Jr.
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