Proceedings of the

# TWENTIETH CENTRAL ALFALFA IMPROVEMENT CONFERENCE

June 22-25, 1987
Champaign-Urbana, Illinois

Reported by
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# TWENTIETH CENTRAL ALFALFA IMPROVEMENT CONFERENCE

June 22-25, 1987

University of Illinois
Champaign-Urbana, IL 61801

Officers:

J. L. Caddel, Chairman, Oklahoma State University
Mark McCaslin, Vice-Chairman, Cal/West Seeds, Wisconsin
Craig R. Grau, Secretary, University of Wisconsin

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TABLE OF CONTENTS

MEETING SCHEDULE
CENTRAL ALFALFA IMPROVEMENT CONFERENCE and NCR-138
Champaign Holiday Inn, Champaign, Illinois
J.L. Caddel, CAIC Chairman, Oklahoma State University
H.W. Kirby, NCR-138 Chairman, University of Illinois

## GENERAL PROGRAM

Monday, June 22, 1987, 7:00-10:00 p.m. Registration and reception at Champaign Holiday Inn.

Tuesday, June 23, 1987

### MORNING

8:00 - 8:30 Registration for late arrivals.
8:30 - 8:45 Welcome and announcements.

### SECTION I. MANAGEMENT AND BREEDING

Chairman - D.A. Miller

8:45 - 9:00 Evaluation of Alfalfa Plant Introductions for Agronomic Traits. D.K. Barnes, D.M. Smith, N.L. Wilson, R.H. Sahi, and N.R. Degenhart, USDA-ARS and University of Minnesota.

9:00 - 9:15 Alfalfa in Tunisia. J.L. Caddel, Oklahoma State University (currently Tunis, Tunisia).

9:15 - 9:30 Plastid Genetics in the Genus Medicago. L.B. Johnson, Kansas State University and J.D. Palmer, University of Michigan.

9:30 - 9:45 Chemistry and Morphology of Alfalfa Cuticular Lipids. J.W. Dillwith, R.C. Berberet, P. Courtney, and A.A. Zarrabi, Oklahoma State University.

9:45 - 10:15 Break

10:15 - 10:30 Allelopathy in Alfalfa and Other Forage Crops. D.A. Miller, University of Illinois.

10:30 - 10:45 Re-establishing Alfalfa after Alfalfa Without Autotoxicity. M.B. Tesar, Michigan State University.

10:45 - 11:00 Drought Response of Alfalfa in Slant-Board Cultures. W. Kendall, USDA-ARS and The Pennsylvania State University.

11:00 - 11:15 Stand Persistence of Alfalfa Cultivars as Influenced by Weeds and Alfalfa Weevil Infestations. A.K. Dowdy, R.C. Berberet, J.F. Stritzke, and J.L. Caddel, Oklahoma State University.

11:15 - 11:30 Modeling the Phenology of the Alfalfa Forage Quality. D.W. Onstad, Illinois Natural History Survey and G.W. Fick, Cornell University.

11:30 - 12:00 Barbecue Lunch at the Agronomy - Plant Pathology South Farm (Courtesy of the Alfalfa Seed Industry)

AFTERNOON

1:00 - 5:00 Tour of Alfalfa Research Projects

EVENING

6:30 - 7:30 Dinner

7:30 - 9:30 NCR-138 Business Meeting and State Reports. H.W. Kirby

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Wednesday, June 24, 1987

MORNING

SECTION II. ALFALFA INSECTS STUDIES
Chairman - R.C. Berberet

8:30 - 8:45 Expression and Heritability of Tolerance to the Spotted Alfalfa Aphid. H.O. Jimenez, J.L. Caddel, and R.C. Berberet, Oklahoma State University.

8:45 - 9:00 Contribution of Erynia spp. for Regulation of Alfalfa Weevil Populations in Oklahoma. K.S. Goh, R.C. Berberet, and L.J. Willson, Oklahoma State University.

9:00 - 9:15 Influence of Alfalfa Dormancy on Population of Alfalfa Weevil and Productivity of Cultivars in Oklahoma. J.L. Reid, R.C. Berberet, and J.L. Caddel, Oklahoma State University.

9:15 - 9:30 Combined Effects of Pest Infestations and Early First Harvest on Alfalfa Productivity in Oklahoma. M.A. Latheef, J.L. Caddel, R.C. Berberet, and J.F. Stritzke, Oklahoma State University.

9:30 - 10:00 Break

SECTION III. ALFALFA DISEASES
Chairman - H.W. Kirby

10:00 - 10:15 Sampling Alfalfa Fields for Plant Parasitic Nematodes. J.A. Thies, D.K. Barnes and A. Peterson, USDA-ARS and University of Minnesota.

10:15 - 10:30 Nematode Species in Illinois and Their Effects on Alfalfa. T.A. Melton, University of Illinois.

10:30 - 10:45 Assessment of Virus Disease Stress on Productivity of Alfalfa. R.G. Gilbert and F. Rahman, USDA-ARS, Irrigated Agriculture Research and Extension Center, Prosser, Washington.

10:45 - 11:00 Mold Quantification Techniques in Alfalfa and Alfalfa - Grass Mixtures. C.A. Roberts, K.J. Moore, D.W. Graffis, and H.W. Kirby, University of Illinois.

11:00 - 11:15 Synergistic and Antagonistic Interactions of Bacteria and Fungi on Roots of Forage Legumes. K.T. Leath, F.L. Lukezic, B.W. Penneybacker, and W.A. Kendall, USDA-ARS and The Pennsylvania State University.

11:15 - 11:30 Interaction of Phoma medicaginis var. medicarginis and Sclerotinia trifoliorum in the Development of Sclerotinia Crown and Stem Rot of Alfalfa. S.D. Mills, L.H. Rhodes, and D.K. Myers, The Ohio State University and Ohio Agricultural Research and Development Center.

11:30 - 11:45 Penetration and Systemic Infection of Alfalfa by Peronospora trifoliorum. D. Trigo Stockli and D. L. Stuteville, Kansas State University.

11:45 - 12:00 Isolate-specific Minor Gene Resistance in Diploid Alfalfa to Peronospora trifoliorum. D.Z. Skinner and D.L. Stuteville, Kansas State University.

12:00 Lunch

AFTERNOON

1:00 - 2:00 CAIC Business Meeting, J.L. Caddel

2:30 - 3:30 Tour of Forage Quality Laboratory, University of Illinois
Forage Cell Wall Analysis - John Fritz
Mold Detection Using NIR - Craig Roberts
Automated Data Collection - Robert Gerhart

3:30 - Start of Post-conference Tour

## POST TOUR ALFALFA RESEARCH AND UTILIZATION AND MARKETING IN ILLINOIS

Tour begins Wednesday evening from University of Illinois at 3:30 p.m. and arrives at Lambert Field, St. Louis by 3:30 p.m. Thursday, June 25.

WEDNESDAY, JUNE 24

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| 3:30 p.m. - 6:00 p.m. | Travel to Springfield, Illinois. We will make motel reservations at Red Roof Inn. |
| 6:30 p.m. | Dinner at Seafood Restaurant or another close by choice. Cost not included in registration. |
| | Van tour of Springfield, Lincoln's Tomb, and Cemetary, Old State Capital and Lincoln Center, |

THURSDAY, JUNE 25

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|---|---|
| 7:30 a.m. | Leave Springfield |
| 9:30 a.m. | Arrive Orr Research Farm |
| 10:30 a.m. | Arrive Oberts Dairy Farm |
| 11:30 a.m. | Arrive Adam Pride, Co., Liberty, IL Alfalfa Cubing |
| 12:15 p.m. | Catered Lunch provided |
| 1:00 p.m. | Leave for Lambert Field, St. Louis Airport |
| 3:30 p.m. | Arrive Lambert Field, Post Tour Adjournment |

# INTRODUCTION

The Twentieth Central Alfalfa Improvement Conference began on June 22, 1987 with registration and a reception at the Holiday Inn in Champaign, Illinois. The following morning registration continued and the conference was convened by Chairman John Caddel at the Holiday Inn.

These proceedings contain reports presented at the conference. The NCR-138 group also met with the 20th CAIC and their reports are included in the proceedings.

We were very fortunate in seeking financial help to help defray expenses for the conference. Listed in the back are 15 commercial seed companies whose contributions sponsored a barbecue lunch and the various coffee breaks. We express our sincere appreciation to all who helped with this event.

Officers for the Twentieth Central Alfalfa Improvement Conference were as follows:

John L. Caddel, Chairman, Oklahoma State University
Mark McCaslin, Vice-Chairman, Cal/West Seeds
Craig R. Grau, Secretary, University of Wisconsin

# Evaluation of Alfalfa Plant Introductions for Agronomic Traits

D.K. Barnes, D.M. Smith, N.L. Wilson, R.H. Sahi and N.R. Degenhart
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During the last decade a national program has been developed for the collection, increase, maintenance, and evaluation of alfalfa germplasm. The current program has been targeted at Plant Introductions (P.I.s). Presently there are more than 2000 alfalfa P.I.s in the U.S. collection. More than 700 of these have been increased in isolation under cage and are now available for the evaluation of agronomic and pest resistance traits. The pest resistance evaluations have been in progress since 1983. Most newly increased P.I.s have been evaluated for resistance to the primary U.S. disease and insect pests. Those evaluations have been conducted at about 8 or 9 different locations. Agronomic evaluation of the alfalfa P.I. collection was originally planned for the Plant Introduction Station at Ames, Iowa. Budget and personnel reductions prevented the completion of those evaluations.

A new USDA alfalfa germplasm evaluation project was initiated at St. Paul, Minnesota in 1986. The project's objectives are to evaluate alfalfa germplasm for resistance to diseases and nematodes; to evaluate alfalfa germplasm for physiological, and morphological traits that affect biological nitrogen fixation (BNF), plant growth, plant adaptation and crop utilization; and to evaluate host-Rhizobium associations as related to germplasm and Rhizobium areas of origin.

The plant growth and morphological characteristics will be evaluated in a two stage program for fall dormancy index, seedling vigor, plant size during the seeding year, summer growth habit, recovery after cutting, crown types, stand survival and yield potential. The first stage will consist of growing single row plots of each P.I. and recording general growth rates and estimating winterhardiness by measuring fall growth (dormancy). The second stage will consist of growing P.I.'s and cultivars in replicated hill plots (7-10 plants/hill). The P.I.'s will be blocked according to plant size and fall growth. Leaf disease, insect damage, etc. will be evaluated if natural epiphytotics or epizootics should occur.

In 1986, 555 P.I.s were evaluated in the first stage of the program. Remarkable variation was observed among entries for summer vigor, erectness, fall growth and winter injury. Extensive measurements were also made on P.I. root systems. Part of each row was undercut at about 25 cm during late October. About 6-10 plants were dug, placed in plastic bags, brought to the greenhouse, washed and photographed. All root scores were made from the photographs. Traits evaluated were determinant tap roots, tap root diameter, number of secondary roots, position of secondary roots, size of secondary roots, and amount of fibrous roots. Significant variability was observed for all types of root systems. A slide set was developed to illustrate the variation available in root traits. This slide set is available upon request.

Present plans include: recording agronomic trait scores for P.I.s in the GRIN system, the evaluation of all U.S. varieties for the same traits, and the exploration of using film to record differences in root and crown traits.

# ALFALFA PRODUCTION IN TUNISIA

John Caddel and Mohamed Chakroun[1]

Tunisia, situation between Libya and Algeria in North Africa, has a Mediterranean climate. Hot summers without rain make alfalfa production possible only with irrigation. Alfalfa's high quality forage is well recognized in this area, but its hectarage is limited to about 50,000 ha because of limited irrigation potential in the country (about 250,000 ha).

Production can be divided into two distinct types: 1) modern, mechanized farms consisting of 50 to 300 ha of alfalfa; and 2) traditional small farms of 1 to 5 ha, utilizing hand labor exclusively. Modern farms are usually government-owned and irrigated with water from deep wells. Traditional farms are normally found among oases in southern Tunisia. Their primary sources of water are springs (or springs and shallow wells combinations).

Alfalfa is marketed or used on the farm as hay, greenchop, or silage. The primary users of alfalfa in the northern part of the country are dairy cattle. In the southern part of the country, traditional farmers profitably market much of their production as fresh green bundles (2.5 kg) for fattening lambs.

Yield and production practices vary widely. Annual yields were reported to be 15 to 30 ton/ha. Stands persist from 3 to 10 years. Small traditional producers try to harvest alfalfa forage prior to bud stage, but the primary criterion is stem height. Mechanized farmers tend to wait until early bloom for harvesting. Alfalfa is sown from September through May; however, November plantings (with barley) appear to be most reliable. Sowing rates tend to be excessive (25 - 35 kg/ha) which may reflect poor seed quality.

Varieties imported from Europe, American, and Australia have had little recognizable impact, and there appears to be little genetic diversity. Nearly all alfalfa in the south is said to be the cultivar 'Gabes', which is very similar to 'African'. Even in northern areas very little cold hardiness is necessary, but types with some dormancy during January and February are found.

No research is currently devoted to alfalfa, thus data on pest problems are difficult to obtain. Pea aphids and spotted alfalfa aphids were easily found in most fields visited in May. At that same time downy mildew was prevalent on stands of all ages. Typical symptoms of Phytophthora root rot were present in fields with drainage problems. In some areas rust was found on almost all plants supporting seed pods. Other production problems mentioned by farmers and extension specialists followed the same lists as for farms in the U.S. or Europe. Additionally, most areas have salty water problems. Salt concentrations in water frequently mentioned were 3 to 4 g/liter and occasionally in excess of 6 g/liter.

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[1]Professor of Agronomy, Oklahoma State University (presently on a 2-year assignment with USAID/MIAC/ATT project) and Forage Agronomist, Institut National de la Recherche Agronomique, Tunis, Tunisia.

# Plastid Genetics in the Genus Medicago

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Smith et al. (5), using chlorophyll-deficient mutants, reported that plastids are biparentally inherited in alfalfa (Medicago sativa L.). Pollen transmission occurred at a very high frequency in the genotypes studied. Leaf sectoring suggested that tissues of mixed plastid type were common. Results obtained by Rose et al. (4) after restriction digestion of cpDNA of protoclones are most easily explainable if protoplast donors were initially heteroplasmic. To further examine the possibility that individual alfalfa plants might contain more than one species of cpDNA, single plant total DNA extracts were made by omitting the sucrose gradient step from the procedures of Palmer et al. (2, 3). DNAs were digested with EcoRV or HaeII or double digested with XbaI and SacI, and the fragments were separated by agarose electrophoresis. Southern filter blots were then hybridized to nick-translated probes (2) of several alfalfa fragments that detect areas of cpDNA polymorphism.

One of two M. sativa ssp. sativa (PI26590) plants and both M. sativa ssp. varia (PI238145) plants showed evidence of cpDNA heterogeneity for a specific XbaI site under conditions yielding complete digestion into two fragments, but none exhibited heterogeneity for HaeII or EcoRV fragments. One of two M. scutellata (PI295606) plants was heterogeneous with both HaeII and EcoRV digests for two fragments that differed as a result of an insertion-deletion event. Plants differing at the XbaI site were probably heterogeneous for cpDNAs, but because the two fragments differed only in the presence or absence of an XbaI site, one cannot eliminate the possibility that another molecule blocked the restriction site and prevented complete digestion. Thus the M. scutellata data are more conclusive. From these results it appears that single plant cpDNA heterogeneity occurs in Medicago, but the frequency of its occurrence will require DNA extraction of progeny from controlled crosses.

In related studies, the chloroplast genomes of a number of accessions of the subgenera Lupularia, Orbicularia, and Medicago in the genus Medicago (1) are undergoing chloroplast DNA restriction mapping with 11 restriction enzymes. These accessions include M. lupulina (PI289310), M. carstiensis (TM993), M. orbicularis (PI385011), M. sativa spp. sativa (PI26590), M. sativa spp. falcata (PI234815), M. sativa ssp. falcata 'Anik', M. cancellata (PI315458), M. dzhawakhetica (UAG98), M. arborea (UAG1497), M. suffruticosa (UAG1545), M. leiocarpa (UAG555) and M. hybrida (UAG2028). As representatives of a genus whose cpDNA lacks an inverted repeat sequence characteristic of all but a few genera of angiosperms in the Leguminosae (2), the evolutionary stability of their cpDNA is of interest (3). Although our results are preliminary, several examples can be given. M.

*sativa* ssp. *sativa*, the two *falcatas* and *M. cancellata* are all quite similar. For example, 'Anik' was detected to differ from spp. *sativa* only in the possession of a small insertion-deletion event and at two restriction sites. In contrast, although mapping of *M. lupulina* cpDNA is still incomplete, it has revealed an 11 kb inversion, over a dozen insertion and deletion events of 0.1 to 2.6 kb, and an even greater number of restriction site changes when compared to spp. *sativa*. Relative to most angiosperm cpDNAs, much rearrangement has occurred. We anticipate using these results in determining phylogenetic relationships within the genus.

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## Chemistry and Morphology of Alfalfa Epicuticular Lipids

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Very little information is available about alfalfa cuticular lipid (wax) structure and composition. Earlier studies (1,2) provided some information about wax chemistry based on available technology and a recent study focused on the amount of wax on plants as a function of environmental conditions (3) and the heritability of wax production. The present study was undertaken to provide a detailed analysis of the cuticular lipids of alfalfa using improved chemical methods and scanning electron microscopy. Four cultivars adapted to different regions of the United States including CUF 101, Riley, Arc and OK08 were used in this study. Plants were maintained under greenhouse conditions and were kept in cages to prevent insect attack and were not sprayed with insecticides.

Scanning electron microscopy revealed that leaves are covered by crystalline wax with a plate type structure characteristic of waxes containing high proportions of primary alcohol. The abaxial and adaxial leaf surfaces showed similar wax distribution and structure. Newly emerged leaves such as the terminal leaf, did not show crystalline wax. Surface waxes were extracted with redistilled carbon tetrachloride according to the method of Galeano et al. (3). Total wax was in the range of 2.5-4.5 mg/gm of wet tissue and 13-23 mg/gm of dry weight. Analysis by thin layer chromatography showed the wax to consist of a mixture of hydrocarbon (9%), wax esters (11%), aldehyde (18%), free fatty acid (<1%) and primary alcohol (61%). The amount of wax present and the wax composition was similar for all cultivars studied. The hydrocarbon fraction consisted of n-alkanes with chain lengths of 25-31 carbons. The wax ester fraction contained several components with chain lengths from 42-52 carbons. Hydrolysis of this fraction yielded a single saturated 30 carbon alcohol and saturated fatty acids with chain length from 12 to 22 carbons. The aldehyde and primary alcohol fraction consisted of a single component with a chain length of 30 carbons. The free fatty acid fraction was made up of saturated acids from 12 to 22 carbons with a chain length of 16 being predominant. The chemical composition of alfalfa cuticular lipid is consistent with established biosynthetic pathways.

Alfalfa possesses a relatively simple cuticular lipid composition. Using this information and procedures established in this study, researchers studying drought and insect resistance should be able to determine if changes in the cuticular lipid composition are related to observed plant properties.

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# Allelopathy in Alfalfa and Other Forage Crops

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Alfalfa producers have observed for many years that they cannot successfully reestablish alfalfa into an existing alfalfa stand that may be beginning to die out (1, 3, 5, 7, 8, 12). We observed this same problem in the late 1960's when we began our annual alfalfa production trial. After three years of continuously reestablishing alfalfa in the same field we observed a reduction in germination, and reduced growth during and after germination. Surveying various alfalfa regions in the U.S., we have found that almost all researchers have observed or found autotoxicity in alfalfa (10). Tesar in Michigan believes there is autotoxicity activity when seeding alfalfa immediately into a former alfalfa field but this activity decreases after two weeks or more (2, 13).

Earlier research indicated that three-day old alfalfa seedlings release toxic compound(s) that inhibit alfalfa 1) germination and 2) seedling elongation (3, 6, 10, 11). It was also found that a relationship exists between 1) radicle elongation and 2) fall growth. A phytotoxic exudate release may play a role in fall dormancy (10). There was no evidence supporting saponins as being the phytotoxic material in alfalfa (11). In addition, there appears to be no major genetic differences among alfalfa cultivars and autotoxicity resistance.

In a greenhouse experiment, it was found that as the plant density increased from 3 to 6 to 9 per $ft^2$ and mixed into an appropriate amount of soil that various plant parts, roots and crowns, or top growth, or whole plant, released phytotoxic with increasing effect, respectively. Percent germination ranged from 80%, for roots and crowns of 3 plants per $ft.^2$ to 62% when 9 plants per $ft.^2$ were mixed into the soil. Similarly when only the top growth was mixed into the soil from 3 to 9 plants per $ft.^2$, germination fell from 47% to 22%, respectively. When the whole plant was mixed into the soil, germination fell from 57% to 20%. Therefore, the greatest amount of allelochemicals are present in the top growth (10).

When top growth of 6, 12, and 18 inches of alfalfa was mixed into the soil, germination and growth was greatly reduced as increasing amounts of top growth was added.

Seedling emergence was significantly higher when alfalfa follows corn in a field rotation as compared to following alfalfa. In another field trial, alfalfa seedling numbers as well as dry matter yields were lower when alfalfa followed alfalfa as compared to alfalfa following other forage grasses.

Allelopathy trials were conducted using smooth bromegrass, orchardgrass, tall fescue, and timothy residue to determine the allelochemical effects on corn, soybeans, and alfalfa production. All grass leaf and root extracts reduced alfalfa germination and radicle length. All grass leaf extracts, but only orchardgrass root extracts reduced soybean germination. All root extracts, but only timothy and tall fescue leaf extracts reduced corn

germination. Alfalfa radicle length was reduced by all extracts, while soybean radicle length was affected most by leaf extracts and corn was unaffected. Tall fescue and smooth bromegrass residue treatments resulted in lower alfalfa populations.

Recently two chemical compounds have been isolated using the GLC and HPLC as medicarpin which inhibits alfalfa germination and 4- methoxymedicarpin which inhibits growth of alfalfa seedlings. Another chemical, ferulic acid is present (4, 9). Other compounds are present and will be isolated in the near future.

## References


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# RE-ESTABLISHING OF ALFALFA AFTER ALFALFA WITHOUT AUTOTOXICITY

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Autotoxicity exists when a plant has lower germination, poorer establishment, and/or lower productivity if grown after itself than after another crop. Autotoxicity in alfalfa (Medicago sativa L.) has been reported in the greenhouse by Henderlong and Li (1981), Jensen et al (1981), and by Miller (1983) in the greenhouse and field. Tesar (1984) found autotoxicity in the field when alfalfa was seeded two days after spraying alfalfa with glyphosate.

Two experiments were conducted between 1984 and 1986 to determine how many days after plowing under or spraying alfalfa with the herbicide Roundup (glyphosate) are compared to establishment after corn. On a 3-year-old stand of alfalfa in late May 1984 (Expt. I) and on a 5-year-old stand in 1985 (Expt. II), Oneida alfalfa was (1) sod seeded 0, 7, 14, 21, and 28 days after spraying to kill the alfalfa or (2) seeded on a tilled seedbed 0, 7, 14, 21, 28, and 195 (fall plow only in Expt. 7) days after plowing under alfalfa. Check plots were seeded at the same time on a tilled seedbed following fall-plowed corn stubble. All seedings were made on the same day and irrigated with one inch of water to minimize differences in germination and emergence.

In the seeding year, yields in both experiments were similar to seeding after corn stubble when (1) seeded on a tilled seedbed 14 days or more after plowing under alfalfa or (2) sod seeded 21 days or more after herbicide spraying of alfalfa.

There were no significant harmful effects of autotoxicity in the year after seeding in the 1984-seeded experiment (Expt. I). In the year after seeding in the 1985-seeded experiment (Expt. II), yields in spring-seeded plots were lower in the alfalfa plowed or sprayed seven days before or on the same day as seeding. When alfalfa was seeded the same day as when sprayed, it yielded 5.17 tons compared to the check (after corn) of 5.62 tons. This was not significant but it indicated some possible autotoxicity if the seeding was made the same day as the old alfalfa was sprayed. In summer-seeded plots (Expt. II), yields were lower than the check only in alfalfa seeded seven days before or on the same day as spraying. Summer-seeded alfalfa, based on these data, is less likely to be injured by any autotoxicity than when spring-seeded. This is possibly due to a greater breakdown of any water-soluble-autotoxicity factor under the higher temperatures of summer compared to spring.

In four variety trials at Michigan State University, the best varieties of alfalfa spring seeded 2 to 3 weeks after spring plowing or after fall plowing of 3-year-old alfalfa stands produced high yields of 8 to 9 tons of hay per year for a 3-year period indicating (but not proving) no significant toxicity if there is a period of two weeks or more between plowing under alfalfa and seeding.

Based on the above research and observations on large fields at Michigan State University and farms in Michigan, recommendations to seed alfalfa after alfalfa without autotoxicity including any appreciable alfalfa yield reduction based on ease of establishment and management are, in priority order:

(1) seeding early in spring on a tilled seedbed after fall plowing of alfalfa;

(2) spring or summer seeding on a tilled seedbed two weeks or more after plowing of alfalfa; or

(3) sod seeding in spring after fall herbicide killing of alfalfa.

The alfalfa variety should be resistant to bacterial wilt Corynebacterium insidiosum (McCull) H.L. Jens., Phytophthora root rot Phytophthora megasperma Drechs f. sp. medicaginis and, if necessary in the area, resistance to Verticillum wilt Verticillum albo-atrum and nematode Pratylenchus penetrans.

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## Drought Response of Alfalfa in Slant-Board Culture

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The objective of this study was to evaluate the use of daily short (ca. 1 hr) periods of plant desiccation as a means of identifying some morphological and/or physiological responses of forage legumes to moisture stress. The moisture stress was achieved by subjecting the entire plant to drying in an air environment as reported by Raynal et al. (2) for plants that grew hydroponically. In our study the plants were grown in slant-board cultures (1) to facilitate exposure of the root system to the drying conditions. Fourteen-day-old alfalfa plants (Medicago sativa L. 'Saranac AR') and red clover (Trifolium pratense L. 'Kenland') were grown at 25-17 C day-night temperature, 15 hr photoperiod, and 250 uE $m^{-2}$ $s^{-1}$ and treated from age 14 to 30 days with 0, 30, or 70 min of drying at ca. 23 C and 60% RH. Plants in the latter treatments occasionally had wilt symptoms beginning to appear, but no extensive wilting occurred in this test.

At the termination of the test, the mean water potential for leaves of alfalfa were -0.73, -0.92, and -1.58 MPa for 0, 30, and 70 min exposure times, respectively. In each species of plants, the desiccation treatments did not affect the dry weight of the roots, but the treated plants had smaller shoots and consequently smaller shoot/root ratios than the treated plants. Further tests with more heavily stressed (wilted) plants appears warranted. As suggested by Raynal et al., advantages of this method are (i) standardization of the intensity and timing of drought, and (ii) avoidance of the confounding influence of mineral nutrient stress.

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# Stand Persistence of Alfalfa Cultivars as Influenced by Weeds and Alfalfa Weevil Infestations

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Many cool season annual weeds grow actively during winter when alfalfa is dormant with little competition until early March when active alfalfa growth begins. Additional stress is imposed on alfalfa plants by the alfalfa weevil (Hypera postica) that hinders the competitive ability of alfalfa. Berberet et al. (1) documented that a compounding effect existed with greatest stand decline where both weeds and alfalfa weevils occurred as compared to areas infested by either pest type individually. Grazing alfalfa in winter has been shown to be successful in reducing or delaying peak alfalfa weevil populations (2,3). However, grazing alfalfa may be detrimental to stand retention by trampling crowns and allowing infection by pathogens. To date, no work has documented the effects of late fall harvesting or winter grazing in combination with weed and alfalfa weevil management on stand persistence in alfalfa.

This study was conducted on an irrigated field planted in the fall of 1981. The experimental design was a split-split plot in strips with four replications of the alfalfa cultivars 'Arc', 'OK08', and 'WL318'. subplots were late fall harvest, winter grazed, or unharvested after September. Sub-subplots consist of a $2^2$ factorial of herbicides (terbacil and oryzalin) and insecticide (carbofuran) resulting in four treatment combinations; alfalfa weevil management only, weed management only, pest free, and unsprayed check plot.

Stand density measurements were made prior to each harvest from five 0.1 $m^2$ quadrats/sub-subplot. Alfalfa weevil larval populations were sampled 3-4 times during the spring to determine the peak population densities. The percentage of weeds in the forage at first harvest was visually estimated and verified with plant separations.

After five seasons of forage production, winter grazing by cattle did not reduce stand persistence relative to alfalfa left unharvested through the winter. There was a tendency for lower stem numbers in the late fall cut management but results were dependent upon cultivar. The rate of stand loss was greatest in OK08 with no pest resistance and least in WL318 which indicates the value of multiple pest resistant cultivars in retaining alfalfa stands for longer periods. By controlling weeds with herbicides for the life of the stand, stem density was 3 stems/ 0.1 $m^2$ greater than in plots with no weed control. Reductions of alfalfa weevil populations below economically important levels (1.5-2.0 larvae/stem) maintained an additional 2 stems/0.1 $m^2$ compared to unsprayed plots. Relative to the pest free treatment (herbicides + insecticide), lower stem densities resulted where either weeds or alfalfa weevils were not managed but greatest losses in stand occurred in combined pest stressed treatments. Late fall harvesting or winter grazing were profitable for utilizing fall regrowth and reducing pest populations the following year without detrimental effects on stand retention.

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# Modeling the Phenology of Alfalfa Forage Quality

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During the past 10 years, our group at Cornell University has been developing phenological models for predicting changes in the protein, fiber and lignin contents and digestibility of alfalfa forage. In this study, a national data set was used to (1) evaluate regression equations previously developed with local NY data and (2) calibrate a new and larger set of models for predicting changes in alfalfa quality. The following six independent variables were tested: age of the canopy in days and degree-days (DD, base of 5°C), sum of the hours of daylight during the growing period, average morphological stage of the canopy (MSW), proportion of leaves by weight, and latitude. Various forms and combinations of the first four variables were included in the older NY equations; all six in the new models. We used stepwise and other regression procedures to discover which simple models (less than four variables) could predict the following five measures of quality: crude protein, neutral-detergent fiber, acid-detergent fiber, lignin, and in vitro true digestibility. The percentage by weight of each factor was predicted for the stem and leaf fractions and the whole canopy.

In the part of the study involving validation of older NY equations, models with MSW as the predictor performed better than those containing other independent variables. At best, logarithmic or quadratic equations with MSW could predict 60-70% of the variability in the quality data. However, predictions of leaf quality had coefficients of determination of 29-45%.

When simple models were calibrated to the national data, MSW again performed, overall, better than any other single predictor. Age of the canopy in days was the best predictor of leaf in vitro true digestibility and second best for leaf crude protein. Equations with DD fit the data for lignin better than other models. This result supports previous studies that concluded that temperature has a major influence on lignin formation. As expected, leaf proportion had a significant effect on quality of the whole canopy. Leaf proportion was included, along with MSW or DD, in all five of the best models for total quality ($R^2$ = 75 to 85%). In the two (out of the possible 15) cases in which it had a significant effect, latitude increased alfalfa forage quality as it increased from south to north. Several of these new functions will be incorporated into Fick's ALSIM models and used in economic analyses of pest management strategies.

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# Expression and Heritability of Tolerance to the Spotted Alfalfa Aphid

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Screening techniques for spotted alfalfa aphid (SAA), Therioaphis maculata (Buckton), resistance were modified with the objective of selecting plants possessing tolerance as the only mechanism of resistance to SAA and estimate its heritability in a common Oklahoma cultivar at the seedling and mature stage. In order for plants to be selected for tolerance, they first must support aphid densities comparable to susceptible plants after 10 days of infestation. Then, plant damage as measured by stem length, stem weight, number of trifoliolates, and number of chlorotic leaves after 19 days of SAA infestations, must be less than for susceptible plants. Tolerance of 48 selected plants was estimated by the expression of each of the above mentioned individual traits and an index was developed with several of the traits included. Broad sense heritability based on stem length, leaf numbers, and numbers of chlorotic leaves in a test of 48 groups of clones was 0.52

After selfed-seed production, 10 progenies from each of the 48 tolerant plants were infested at the unifoliolate stage for 12 days. Average stem length and numbers of trifoliolates produced by the seedlings were regressed on the values for the 48 parental plants which had been measured at the same stage of growth after 10 days of SAA infestation. The regression coefficient estimated the proportion of the phenotypic variation of the progenies that could be explained by the genotype of the parent (1). For the damage index (stem length + numbers of trifoliolates) analysis, the coefficient of regression was 0.83, which is an estimation of heritability in the broad sense considering that dominant effects could remain by self-fertilization (2).

Polycrossed progeny were also obtained by hand pollination among all 48 selected plants. Ten seedlings from each of the 48 polycrossed progenies (half-sibs) were tested by 12 days of SAA infestation. Average stem length and numbers of trifoliolates produced by the progenies were regressed on the values of the parental plants. Twice the regression coefficient estimated the heritability of tolerance to the SAA. The regression coefficient for stem length plus number of trifoliolates was 0.24, which indicated a heritability of 0.48. Because of low dominant effects passed to the polycrossed progenies, this heritability could closely estimate the proportion of additive variance to the phenotypic variance (narrow sense heritability) (1). Expression of tolerance to SAA through better stem growth only, had a heritability of 0.16 in the polycrossed progenies.

One to two seedlings were selected within each polycrossed family to start a second cycle of Selection. This second cycle of polycrossed progeny was tested and indicated a realized heritability (actual gain) of 0.41 based on measurement of stem length after 12 days of SAA infestation.

These studies indicated that tolerance to SAA is substantially controlled by dominant effects as shown by the high degree of heritability in the broad sense and low degree in the narrow sense. Realized heritability for two cycles of selection was intermediate, which suggested that some dominant effects could still remained in the second polycrossed progeny.

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## Contribution of *Erynia* spp. for Regulation of Alfalfa Weevil Populations in Oklahoma

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The fungal pathogen, *Erynia* spp. was first reported in the alfalfa weevil, *Hypera postica* (Gyllenhal) by Harcourt et al. (1) in Canada. High mortality due to this pathogen in weevil populations has prompted several studies in the United States (2,3,& 4). In Oklahoma, *Erynia* spp. was observed for the first time in 1983. Fungal epizootics have usually been detected from late March to early May. Our study was done to determine the extent of mortality on a seasonal basis in the weevil population due to infection by *Erynia* spp.

Data were collected from 1983 to 1986 on the Oklahoma Agricultural Experiment Station at Stillwater (Payne Co.). Larval populations were estimated by collecting 10-0.1$m^2$ samples of alfalfa foliage of each six-eight sampling dates during March, April and May of each year. Larvae were extracted using Berlese funnels and counted by instar. In addition, larvae, prepupae, and pupae were randomly collected and reared individually in vials until adult emergence or occurrence of death. The presence of the fungus in cadavers was confirmed by making wet mounts for microscopic examination.

Two types of cadavers were observed. Tan-colored cadavers contained mycelia from which conidiophores were produced, and black and shrivelled cadavers contained resting spores. Both types of cadavers occurred in larvae whereas infected prepupae and pupae generally contained resting spores.

Fungal infections usually occurred following peak weevil populations. Rate of infection in larvae generally ranged from 35 to 100%. The fungal pathogen appeared to infect third and fourth instars much more often than smaller larvae. Fungal infections of prepupae ranged from an average of 45 to 90%, whereas infection in the pupae was less than 40%.

As a natural control agent, *Erynia* spp. appeared to be effective in eliminating weevil populations during epizootics following peak larval densities. However, it was not effective in preventing economic damage to alfalfa prior to occurrence of peak infestations.

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# Influence of Alfalfa Dormancy on Alfalfa Weevil Populations and Productivity of Cultivars in Oklahoma

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Yield losses occurring in alfalfa can be attributed to several diseases, multiple pests, and various environmental stresses. One serious insect pest damaging Oklahoma alfalfa fields is the alfalfa weevil. If heavy weevil infestations occur prior to first harvest, it has been shown that alfalfa growth, yield, rate of maturity, and stand density can be reduced during the season (1,2). However, damage by the alfalfa weevil has been reported to be less for taller, more rapid growing alfalfa (3); thus, yield reductions may not be as great. Alfalfa dormancy may regulate growth characteristics which could influence weevil infestations. Therefore, the objectives of this study were to compare the productivity of dormant, moderately dormant, and nondormant alfalfa cultivars under weevil infested and uninfested conditions.

Field plots were established at the Agronomy Research Station at Stillwater, Oklahoma in September 1984. The five cultivars selected were Advantage and WL 318-(dormant), Baron and WL 515-(moderately dormant), and CUF 101-(nondormant). These cultivars were grown under weevil infested and uninfested treatments in a randomized complete block design with 6 replications. Alfalfa weevil populations were controlled the seedling year. Natural weevil infestations were allowed to build up during 1985/1986 in the weevil infested plots only. Weevils were controlled with carbofuran at peak larval infestation in the infested plots and at first sign of damage in the uninfested plots. Larvae were sampled in March and April 1986 from 30 stems/plot. Effects of larval feeding were determined by visually rating each plot for feeding damage using a 1 to 9 scale. Plant height of 10 stems/plot were also measured. Harvest occurred at approximately 10% bloom throughout the 1986 season. Crude protein was estimated by 1 subsample taken from each plot.

Larval hatch began early in the season due to extremely mild temperatures that occurred in the 1986 winter. By March 21, the nondormant cultivar, CUF 101, had a significantly greater larval density. No differences in damage were evident at this time. By April 4th, peak larval hatch had occurred with no significant differences in larval numbers among the cultivars. The two dormant cultivars, Advantage and WL 318, faired better under the infested treatment as they had significantly lower damage ratings.

Five cuttings of alfalfa were harvested from April through September 1986. Significant differences between yields from weevil infested and uninfested plots occurred through the 4th harvest. It was not until the 5th harvest that the residual effect of early weevil damage was no longer displayed in yield. There was no treatment X cultivar interaction in any harvest.

Effect of alfalfa weevil damage was most evident from the 1st harvest. Cultivars in the weevil infested plots produced only about 1/2 the yield when compared to the uninfested plots. In the weevil infested plots, Advantage, WL 318, and Baron had significantly higher yields than CUF 101. Advantage

clearly had the superior yield in the uninfested plots, producing 6.0 M Tons/ha.

The alfalfa weevil had a significant effect in stunting growth before 1st harvest. Alfalfa in the uninfested plots was over twice the height of that in the weevil infested plots. The two dormant cultivars in the infested plots were affected less by alfalfa weevil feeding than the nondormant. Dormant and moderately dormant cultivars maintained comparable heights in the uninfested plots.

Cultivars in the weevil infested plots at 1st harvest displayed gradual decreases in % crude protein as dormancy level decreased. The two dormant cultivars had a significantly higher crude protein than the nondormant cultivar, CUF 101, and they performed very well under weevil infestation. All cultivars in the uninfested plots produced high crude protein levels.

Total yield for cultivars in the weevil infested plots were 5-7 M Tons/ha less than those in the uninfested plots. Both dormants and the moderately dormant, Baron, performed significantly better under weevil infestation. The nondormant cultivar, CUF 101, produced less forage in both treatments. This reduction probably represents a decrease in plant vigor due to winterkilling and periods of standing water prior to first harvest.

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## Combined Effects of Pest Infestations and Early First Harvest on Alfalfa Productivity in Oklahoma

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Reductions in yields and stand densities in alfalfa due to damage by the alfalfa weevil (Hypera postica) has been documented in many studies (1,3). Recent studies have shown that, in the absence of insect infestations, early first harvest (early bud) does not cause losses in productivity of stands (2), Latheef et al. (unpublished). Our research was designed to study possible interactions of alfalfa weevil damage and first harvest timing with the objective of determining whether a particular harvest schedule in combination with the weevil damage resulted in an additive effect for yield and/or stand reductions.

The cultivars 'WL-318', 'Arc', and 'OK08' were planted in fall of 1981 with harvest and pesticide treatments arranged in a split-split-plot design first imposed in 1982. In our first study, cultivars were main plots, sub-plots were either sprayed (carbofuran insecticide + terbacil and oryzalin herbicides) or unsprayed, and harvest times (early bud, late bud, early bloom, or late bloom) were on sub-sub-plots. Subsequent harvests were taken at 10-40% bloom with intervals altered sufficiently to bring all treatments into synchrony for the last cut in September. In 1985 all plots were treated with insecticide and harvested at the same times to permit analysis of residual treatment effects. Samples of 25 stems/sub-sub-plot were taken during the first crop growth for alfalfa weevil counts. Percent ground cover by alfalfa plants was estimated in February of each year. Plants were undercut and counted in a 5 $m^2$ area of each sub-sub-plot in March of 1986.

A second experiment was begun in 1986 in fifth year stands of WL-318 and OK08. In this split-split-plot design, main plots were cultivars, while insecticide applications for weevil-free, moderate infestation, vs. unsprayed were randomized on sub-plots, and first harvest times (early bud, early bloom, late bloom) were on sub-sub-plots.

Peak weevil populations in unsprayed plots ranged from 2-3 larvae/stem in 1983 and 1984 prior to first harvest. The damage resulted in significant ($p < 0.05$) yield reductions for some first harvest treatments, but no consistent trends occurred. As expected, first harvest yields were lowest for the early bud treatment in both sprayed and unsprayed plots. In most instances, there were no significant differences ($p > 0.05$) in yearly yield totals for either sprayed or unsprayed treatments. There was no consistent trend for lower yield totals for any first harvest timing. Measurement of residual effects in 1985 showed significant ($p < 0.05$) reductions in the yearly total yield for unsprayed alfalfa with all first harvest treatments. Percent ground cover was also lower for all unsprayed treatments. Differences in yields among harvest times were minimal for both sprayed and unsprayed alfalfa.

Weevil populations were much higher during experiment two with peak densities up to 10/stem in unsprayed plots. Unsprayed alfalfa had

significantly ($p < 0.05$) lower first harvest and yearly total yields in 1986 than that with at least some weevil control. Total yield was significantly ($p < 0.05$) lower for early bud harvest than late bloom in the weevil-free treatment. Stand densities (stems/0.1 $m^2$) were lower in unsprayed than in weevil-free plots for all harvest treatments in April of 1987. Significant ($p < 0.05$) reduction in yields occurred in all harvest schedules due to weevil damage.

In summary, relatively consistent reductions in yield and stand of alfalfa have occurred with alfalfa weevil infestations in all first harvest treatments. We have no results to-date that indicate a compounding effect of any first harvest schedule and weevil infestation.

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## Sampling Alfalfa Fields for Plant Parasitic Nematodes

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Plant parasitic nematodes typically occur in clumped or aggregated horizontal patterns. Nematodes are located in the root zone and their distribution varies with soil type and texture, soil drainage, and direction of cultivation. Season, soil moisture and temperature, and crop species also influence nematode distribution.

Sampling objectives should be defined prior to collecting samples in order to determine the sampling design and the number of samples needed to accurately estimate the numbers of nematodes present (1.). Sampling objectives include: survey, diagnostic, advisory, and research. One sample per field is usually adequate for survey purposes. Diagnosis requires sampling both healthy and diseased plants. Sampling for advisory purposes requires that areas of differing soil type, texture, and drainage be sampled separately. Sampling design, sample type (soil or roots), sample timing, and number of samples are dependent on the reserach objectives and experimental design.

A polyspecific community of ectoparasitic and endoparasitic nematode species may require sampling both soil and roots. Soil cores should be collected in the root zone to a depth of 25 cm with a 25 mm diameter sampling tube. Roots should be sampled by digging individual plants with surrounding soil.

In June 1986, two alfalfa fields at Rush City, MN which were infested with root-lesion nematodes (Pratylenchus spp.) were sampled for the purpose of studying nematode distribution patterns. The objectives of this study were: to determine the relationship of numbers of root-lesion nematodes in alfalfa and quackgrass roots; and to relate root-lesion nematode numbers in plant roots with soil elevation, texture, ph, percent organic matter, P and K characteristics. The fields were divided into grid sections approximately 0.13 $ha^2$. Ten plant samples were systematically collected within a 7 meter diameter circle in each section. Nematodes and eggs were stained within the roots and counted. Nematode + egg numbers were plotted on the map, but nematode distribution patterns were not evident, so the field was surveyed, a topographic map was made, and soil drainage patterns were mapped.

Elevation was negatively correlated with percent organic matter and soil texture ($r = -0.47$ and $-0.56$, respectively, 12 d.f.). Percent organic matter was correlated with soil texture and K ($r = 0.66$ and $0.71$, respectively, 12 d.f.). Numbers of nematodes + eggs were negatively correlated with ph and positively correlated with K ($r = -0.52$, 20 d.f., and $r = 0.72$, 12 d.f., respectively). Numbers of nematodes + eggs in alfalfa and quackgrass roots were correlated ($r = 0.84$, 12 d.f.). Numbers of nematodes + eggs $g^{-1}$ fresh root quackgrass, bromegrass, bluegrass, and clover spp. were equal to or greater than in alfalfa roots.

Conclusions: Numbers of root-lesion nematodes in alfalfa and quackgrass roots were correlated. The relationship of root-lesion nematode distribution to soil characteristics is complex. However, nematode distribution patterns appeared to be related to soil drainage patterns.

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## Assessment of Virus Disease Stress on Productivity of Alfalfa

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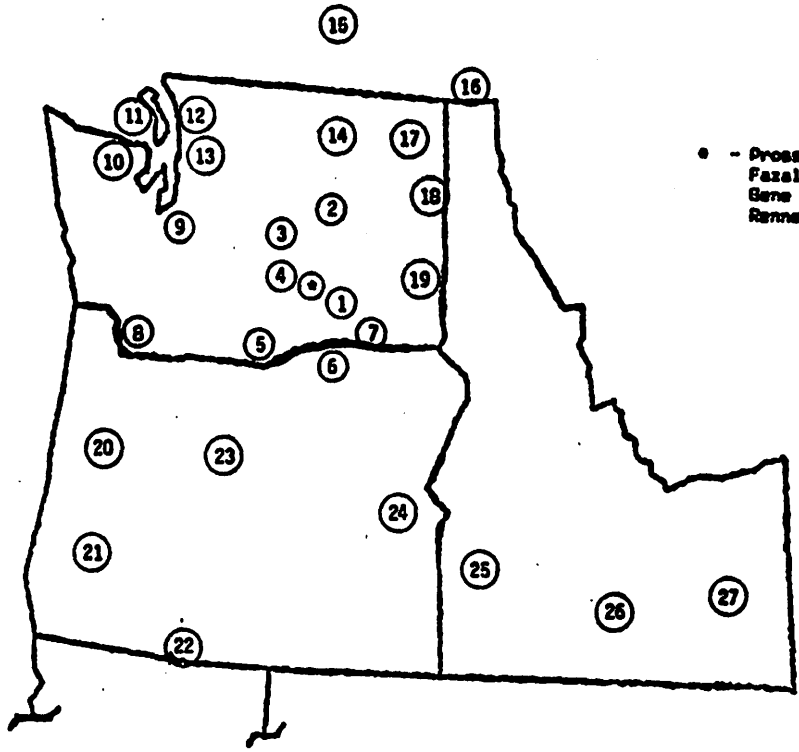
It has been well established that alfalfa serves as a reservoir for several viruses that are infectious to other legumes, especially beans and peas. However, virtually nothing is known as to the range of viruses that alfalfa hosts or if alfalfa is affected by these viruses. Therefore, alfalfa virus research was started that included surveying and identifying the predominant viruses in alfalfa from the Pacific Northwest. The virus survey presently includes 28 sites ranging from Penticton, B.C., Canada, to Klamath Falls, OR, and from Sequim, WA, to Pocatello, ID. Five to 10 alfalfa fields in their third year of production were selected from each site. Ten samples of young shoot tip tissue on a preestablished grid pattern (about 10 random shoot tips/sample) were placed in plastic bags and stored on ice. Individual plants from each field showing virus symptoms were also collected in a similar manner. Upon return to the laboratory, all samples were stored in a refrigerator (4°C) until they were diced up with razor blades and the tissue was dried over "Drierite". These dried alfalfa samples may be stored in a refrigerator for an indefinite period. All samples will be routinely assayed by ELISA procedures against seven viral antisera: 1) alfalfa mosaic virus (AMV); 2) alfalfa latent/pea streak virus (PSV); 3) pea enation mosaic virus (PEMV); 4) pea (bean) leaf roll virus (PeLRV); 5) red clover vein mosaic virus; 6) tobacco streak virus (TSV), and 7) antipotyvirus. Selected symptomatic samples and individual or composite samples from each field site will be assayed on biological indicator hosts to better characterize and identify the predominant viruses. Also, electron microscopy will be used for further delineation and identification of unknown virus isolates. The early results with biological assays of samples from the Prosser, WA, site have indicated the presence of 10 distinct viruses or virus strains that have been subcultured on various indicator hosts and stabilized. These include the following: AMV, PSV, PEMV, TSV, and cucumber mosaic virus (CMV). Numerous symptoms on various host plants still need to be identified with specific viruses. Additional results that become available from the on-going survey will be presented for discussion. Subsequent cooperative USDA-WSU research is planned to evaluate the influence of endogenous virus infections in alfalfa on photosynthesis and primary productivity, on interactions with other plant pathogens, on selection of disease resistant germplasm, on root nodulation by *Rhizobium* and nitrogen fixation, and on agronomic aspects of alfalfa, such as dormancy, regrowth, stand life, forage yield and quality, and seed production.

SURVEY OF VIRUSES FROM ALFALFA IN THE PACIFIC NORTHWEST:
SAMPLE SITES (Numbers 1 to 27)




* - Prosser, Wa. (Benton); 509-786-2484
Fazal Rahman
Gene Gilbert
Ranna Soberg

1 - Pasco, Wa. (Franklin); 509-545-3511
Bill Ford

2 - Ephrata, Wa. (Grant); 509-754-2011
John Moore

3 - Ellensburg, WA. (Kittitas); 509-962-6811
Charlie McKinney

4 - Yakima, Wa. (Yakima); 509-575-4242
Jim Griffin

5 - Goldendale, Wa. (Klickitat); 509-773-5817
John Fouts

6 - Hermiston, Or. (Umatilla); 503-567-8321
Luther Fitch

7 - Walla Walla, Wa. (Walla Walla); 509-527-3260
Walt Gary

8 - Vancouver, Wa. (Clark); 206-699-2385
Gary Fredrick

9 - Puyallup, Wa. (Pierce); 206-593-8540
Steve Fransen

10 - Sequim, Wa. (Clallam); 206-452-7831
Jack Waub

11 - Copeville, Wa. (Island); 206-679-7327
Don Meehan

12 - Bellingham, Wa. (Whatcom); 206-676-6736
David Grusenmeyer

13 - Mt. Vernon, Wa. (Skagit); 206-336-9322
Everett, Wa. (Snohomish); *206-338-2400
Richard Mathews

14 - Okanogan, Wa. (Okanogan); 509-442-3670
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15 - Penticton, BC (Canada); 604-498-6235
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Bryan Laing

17 - Colville, Wa. (Stevens); 509-684-2588
Wayne Madson

18 - Spokane, Wa. (Spokane); 509-456-3651
Paul Peterson

19 - Pullman, Wa. (Whitman); 509-397-3401
John Burns
Walt Kaiser (509-335-1501)
Otis Malloy (509-335-2965)

20 - Corvalis, Or. (Benton); 503-967-3871
Mark Mellbye and Gary Stevenson
Paul Koepsell (503-754-3472)
Dick Hampton (503-754-3451)

21 - Roseburg, Or. (Douglas); 503-672-4461
Paul Friedrickson

22 - Klamath Falls, Or. (Klamath); 503-883-7131
Rodney Todd

23 - Redmonds, Or. (Deschutes); 503-548-6088
Marvin Young

24 - Ontario, Or. (Malheur); 503-881-1417
Ben Simko

25 - Caldwell, Id. (Canyon); 208-454-7461
Darrell Bolz
Bob Romanco (208-459-6365)

26 - Twin Falls, Id. (Twin Falls); 208-734 9590
Bill Hazon
Truman Massey (208-423-5582)

27 - Pocatello, Id. (Bannock); 208-236-7310
George Gardner

## VIRUSES DETECTED WITH BIOLOGICAL INDICATOR HOSTS

ALFALFA MOSAIC VIRUS

PEA STREAK VIRUS

PEA ENATION MOSAIC VIRUS

TOBACCO STREAK VIRUS

CUCUMBER MOSAIC VIRUS

UNKNOWNS (5 Subcultures)

## BIOLOGICAL INDICATOR HOSTS

Cowpeas

Tomatoes

Squash

Cucumbers

Beans

*Gomphrena globosa*

*C. amaranticolor*

*C. quinoa*

*Amaranthus retroflexas*

*Amaranthus Caudatus*

*Capsicum frutescens*

Peas

Spinach

True Irish Clover

Red Clover

*Tobacco xanthi*

*N. Tobaccum*

*N. Tobaccum* 423

*N. Glutinosa*

## LIST OF "ELISA" VIRUSES

ALFALFA MOSIC VIRUS (AMV)

PEA STREAK VIRUS (PSV)

PEA ENATION MOSIC VIRUS (PEMV)

PEA LEAF ROLL VIRUS (PeLRV)

RED CLOVER VEIN MOSIC VIRUS (RCVMV)

TOBACCO STREAK VIRUS (TSV)

ANTIPOTYVIRUS

# Mold Quantification Techniques in Alfalfa and Alfalfa-grass Mixtures

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The inability to accurately determine fungal contamination in hay has affected alfalfa research and hay marketing. In research efforts, results of hay preservation studies have been frequently based upon subjective, visual assessment or spore counts. In electronic marketing, hay evaluation has been incomplete because only quality components have been addressed.

The lack of fungal assessment in food products has been resolved by the chemical determination of chitin, a fungal cell wall constituent. Chitin has been used to quantify mold in many grains, such as corn, soybeans, barley, and wheat (2,3,10) as well as many fruit and vegetable products (1,4,7,8). If these procedures could be used to chemically quantify mold in hay, the data could possibly be used to spectrally predict mold with near infrared reflectance spectroscopy (NIRS). This study was conducted in order to quantify mold in alfalfa (*Medicago sativa* L.) and alfalfa-grass mixtures by the chemical and spectral determination of chitin and to compare chitin data with spore counts and visual estimation.

In study I, alfalfa and alfalfa-grass samples were collected from individual producers in Illinois. Samples were analyzed for spore count and chitin (8). The spore count procedure involved suspending a 0.5g subsample in $H_2O$:tween 80 (30:1 v:v) and mixing thoroughly. After large particles settled, spores were counted under microscopic examination of 40 fields of a hemocytometer (6).

Spore count was not correlated ($p>.05$) to chitin. The lack of a relationship between mold count and chitin is not uncommon and is primarily attributed to error associated with both sample preparation and mold count procedures (1,4). While it is true that subsampling error in the spore count procedure may have been reduced with other techniques, such as a wind tunnel (6), it is doubtful that the 0.5g subsample could have been more representative of the sample because the samples were small (20 - 50g) and mixed very well. In this study, the lack of a relationship between the microscopic and chemical techniques could have been influenced by endophytic chitin in perennial ryegrass (*Lolium perenne*) and tall fescue (*Fescue arundinacea* Schreb.); it has been shown that chitin from samples infected with *Acremonium coenophialum* often exceed 500 µg/g DM (9) and has probably interferred with the chemical estimation of post-harvest mold of tall fescue hay (5).

In study II, alfalfa samples collected from Wisconsin and Illinois were analyzed for chitin. Core samples from Wisconsin had been visually evaluated using a relative mold index (RMI). Criteria for RMI were as follows: 1 = no visible mold, 2 = presence of dust or spores between flakes, 3 = dust throughout entire bale, 4 = myelial mat between flakes, and 5 = mycelial mat through entire bale. Mycelial cell walls were quantified by chitin determination. Near infrared spectra were collected from all samples and a calibration equation was developed by regressing reflectance spectra against chemical data.

Chitin concentrations ranged from 75 to 710 µg/g dry matter (DM) and were similar to concentrations in infected fruit, grain and vegetable products (1,2,3,4,7,8,10). The

coefficients of determination for calibration and validation were .90 and .82, respectively. The standard errors of calibration and validation were 44 and 65 µg/g DM, respectively. The large standard errors were attributed both laboratory precision and instrument sensitivity because chitin concentration was minute. The calibration equation utilized 4 terms, represented at wavelengths 1630, 2114, 2246, and 2356 nm. The validated equation was used to predict mold (chitin) in bales which had been visually estimated for mold level with a relative mold index; RMI was regressed against NIRS-predicted chitin and was correlated to chitin (r = .86). Residual error was small when chitin was extreme.

We concluded from study I that the number of spores in infected alfalfa and alfalfa-grass hay was not consistently related to mycelial dry matter. We concluded from study II that chitin was representative of mold contamination in alfalfa and that NIRS could accurately predict chitin.

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## Synergistic and Antagonistic Interactions of Bacteria and Fungi on Roots of Forage Legumes

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Various interactions between bacteria and pathogenic fungi were evaluated for antagonistic or synergistic effects on root disease development. Several inoculation techniques, involving wounding, were used on roots of red clover and alfalfa grown hydroponically under growth-chamber or gnotobiotic conditions. Inoculum consisted of bacterial slime or cell suspensions and fungal mycelial or conidial suspensions. Macroscopic and microscopic examinations indicated an increase in disease severity when Fusarium avenaceum and Pseudomonas viridiflava cohabited the root tissue. Periderm, phloem, vascular cambium, xylem parenchyma, fibers, and occasionally xylem vessel elements were disintegrated by these organisms acting together. However, only limited tissue destruction was caused by either organism independently.

The interactions of Bacillus subtilis with several root and seedling fungal pathogens of alfalfa and red clover were evaluated in a gnotobiotic system. The bacterium reduced the activity of Colletotrichum trifolii and has potential as a biocontrol agent against diseases of forage crops. Phytotoxicity of a B. subtilis preparation, however, has occurred in some applications. Root disease symptoms caused by a Pythium sp. on alfalfa were less severe in the presence of B. subtilis, but the reduced disease severity was not evident in increased plant dry weight.

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# Penetration and Systemic Infection of Alfalfa by Peronospora Trifoliorum

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Reports vary on the mode of penetration of alfalfa (Medicago sativa L.) leaves by conidia of Peronospora trifoliorum d By., the downy mildew fungus. Waite and Cannon (5) studied penetration by P. trifoliorum at 5 and 15 C and reported that germ tubes penetrated only through stomata. However, Rockett (4), working at 20 C, noted that P. trifoliorum germ tubes formed appressoria and penetrated directly between the epidermal cells. As Cohen (1) showed that temperature affects the method of germ tube penetration by some Peronosporales, we studied penetration at four temperatures by three pathogenically different isolates of P. trifoliorum. Alfalfa plants resistant and susceptible to the P. trifoliorum isolates used, were inoculated at 5 and 19 days after seeding by spraying with conidia suspended in water and were incubated at 10, 15, 20, and 25 C in darkness and in light. At least 200 germinated conidia were observed per treatment.

By 12 hr after inoculation, every conidial germ tube produced an appressorium. Approximately 95% penetrated between epidermal cells and about 5% penetrated stomata. Mode of penetration was not affected by temperature or isolate used, by age of inoculated leaves from susceptible or resistant alfalfa plants, or by light or darkness during the germination and infection period. Scanning electron microscopy showed that P. trifoliorum germ tubes formed appressoria in grooves in the anticlinal wall surface of leaf epidermal cells.

In the temperate regions, systemically infected alfalfa plants are an important means of overwintering by P. trifoliorum and likely provide most of the early spring inoculum (3). Jones and Torrie (3) noted that only a portion of susceptible alfalfa plants became systemically infected. Hodgden (2), while studying some environmental effects on P. trifoliorum oospore production in alfalfa seedlings, observed that the proportion of plants with systemic symptoms was greatest at 12 C, the lowest temperature used, and under continuous light at the highest intensity used. To gain additional information, we studied the effects of host age and temperature on systemic infection. We used the $S_1$ progeny of alfalfa plant 275-1 because they are highly and uniformly susceptible to all P. trifoliorum isolates that we have tested. Seeds were planted 6, 13, 20, and 27 days prior to inoculation and placed in a growth chamber at 20 C and a 24-hr photoperiod of 72 $\mu E \cdot m^{-2} \cdot s^{-1}$ light intensity. After inoculation with a conidial suspension of P. trifoliorum isolate I-5, the plants were kept in dark at 20 C for 24 hr to permit infection and were then transferred to growth chambers at 10, 15, 20 or 25 C with continuous 72 $\mu E \cdot m^{-2} \cdot s^{-1}$ cool white fluorescent lighting. The percentages of plants with systemic symptoms of downy mildew were recorded 7, 14, 21, and 28 days later.

The younger the plants at inoculation the greater the percentage that developed systemic symptoms at each of the four temperatures (Fig. 1). However, the percentage of plants at each age that developed systemic symptoms, and the length of time they retained them, tended to decrease as temperature increased. No plants at 20 or 25 C had systemic symptoms 28 days after inoculation.

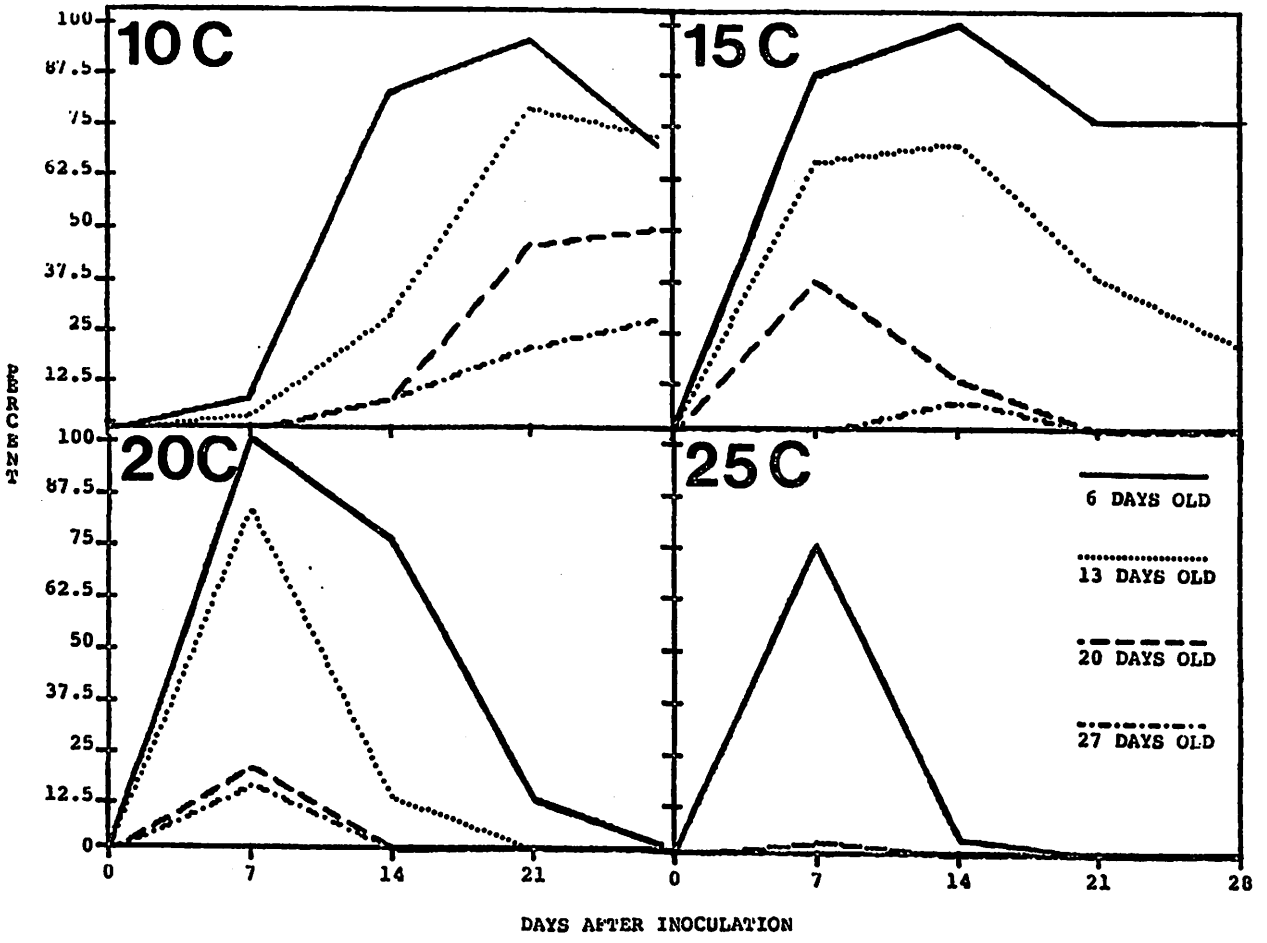


Fig. 1. Effects of alfalfa plant age at time of inoculation with Peronospora trifoliorum conidia and postinoculation temperatures on the percentage of plants with systemic symptoms 7, 14, 21, and 28 days after inoculation.

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# Isolate-Specific Minor Gene Resistance in Diploid Alfalfa to Peronospora trifoliorum

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Our previous investigations with diploid Medicago sativa L. plants indicated that in some plants dominant major resistance genes individually prevented conidial production by Peronospora trifoliorum d By., the causal fungus of alfalfa downy mildew (1). Those genes were expressed only in response to certain fungus isolates (1), and therefore were isolate-specific major genes.

We also found many genes involved with cumulative effects (2). These "minor genes", in certain combinations completely inhibited conidial production (2). Preliminary results (3) suggested that the effects of these "minor genes" were general, with respect to the three fungus isolates that we tested. General resistance is valuable from a breeding perspective because it is not overcome by new genotypes of the fungus.

The objectives of this study were to further investigate the possibility that minor gene resistance in this pathosystem was general in effect, to examine the expression of minor gene resistance in the progeny of a resistant by susceptible plant cross, and to determine if plants with only minor gene resistance could be developed which produced only resistant $S_1$ plants.

Parental material for this study was diploid alfalfa plants P1 and P2 which expressed no major genes for resistance to P. trifoliorum isolates I-5, I-7, or I-8 (1). We selected only for resistance to I-7, and those plants whose $S_1$ progeny were most resistant were used in subsequent crossing cycles shown on Table 1.

All $S_1$s of plant P1 supported uniformly profuse sporulation, whereas 3% of the $S_1$s from plant P2 were resistant (did not support sporulation) to isolate I-7. $F_1$ plants of P1 and P2, (P1 X P2)A and (P1 X P2)K, whose $S_1$ populations were 59% and 37% resistant to I-7, respectively, were crossed. Then, two of their progeny, (A X K)B and (A X K)E, whose $S_1$ populations were 73% and 76% resistant to I-7 were crossed, and resistant progeny were selected. High levels of minor gene resistance were expressed to isolate I-7, and also to pathogenically different isolates I-5 and I-8 in the $S_1$s of progeny of the second cycle of selection (Table 1). This result suggested that some of the minor gene resistance was general in effect. However, when plants (A X K)B and (A X K)E were crossed and their progeny evaluated for resistance to I-7, some resistant $F_1$ plants produced $S_1$ populations largely resistant to I-7, but much less resistant to I-5 and/or I-8 (Table 1). Plant (E X B)B produced an $S_1$ population 73%, 98%, and 58% resistant to I-5, I-7, and I-8, respectively. However, when (E X B)B was backcrossed to P1, the resulting population was 9%, 14%, and 0% resistant to isolates I-5, I-7, and I-8, respectively.

These results suggested that the apparent general resistance was at least partially, and probably entirely, due to a collection of genes with isolate-specific effects. Therefore, minor gene resistance in this pathosystem may not endure in a field situation. The result of the (E X B)B X P1 backcross also suggested that minor gene resistance was isolate specific, and usually was not expressed in the progeny of a resistant by susceptible plant cross. Therefore, using minor gene resistance in the improvement of susceptible breeding lines would require careful selection for resistance from a large population.

Table 1. Reactions to three isolates of Peronospora trifoliorum by $S_1$ populations of diploid alfalfa plants selected for resistance to isolate I-7

| | % of $S_1$ plants resistant[a] to isolate | | |
|---|---|---|---|
| Plant[b] | I-5 | I-7 | I-8 |
| P1 | 0 | 0 | 0 |
| P2 | 4 | 3 | 1 |
| (P1 X P2)A | 26 | 57 | 37 |
| (P1 X P2)K | 29 | 37 | 21 |
| (A X K)B | 56 | 73 | 70 |
| (A X K)E | 67 | 76 | 50 |
| (E X B)B | 73 | 98 | 58 |
| (B X E)3 | 22 | 82 | 13 |
| (B X E)7 | 35 | 93 | 47 |

[a]Resistant = did not support conidial production.
[b]Plants above a line of dashes were the parents of the plants immediately below that line.

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# TOUR:
# University of Illinois Forage Quality Laboratory

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## Automated Data Collection

One objective of the University of Illinois Forage Quality Laboratory is computer-interfaced data aquisition. Electronic data aquisition reduces the opportunity for error because it eliminates manual recording onto data sheets and posting into spreadsheets. Current automation in this laboratory is used to aquire gravimetric data with a Mettler AE 160 interfaced with an IBM PC. Chromatographic data is also aquired electronically from a Hewlett-Packard 5890 gas chromatograph linked to an IBM PC. The required software is either privately developed or commercially obtained (Lotus Measure). Commercial software allows for direct interaction with Lotus 1-2-3; therefore, tedious manipulation of ASCII files is minumized. Future automation will extend to a UV-vis spectrophotometer and will be used for colorimetric analysis of carbohydrates, chitin, nitrogen, and uronic acids.

## Cell Wall Chemistry

Detergent System. The detergent system of forage fiber analysis, developed primarily by Van Soest, allows the fractionization of fiber into its major components. Extraction with neutral detergent solution removes the highly digestible and soluble components of forage dry matter and allows recovery of the structural or cell wall components cellulose, hemicellulose and lignin. The residue recovered after neutral detergent extraction is often referred to as neutral detergent fiber (NDF) and is highly correlated to feed intake by ruminant animals. Extration of the cell wall with acid detergent solution (1 N sulfuric acid) removes and allows estimation of the hemicellulose. The remaining residue or acid detergent fiber (ADF) is composed of cellulose and lignin and is highly correlated to the digestibility of a feed. Treatment of ADF with 72% sulfuric acid removes cellulose and allows the isolation of crude lignin. Estimation of lignin is important as it is not degraded by rumen microorganisms and is known to limit the digestibility of the polysaccharide components of the cell wall (cellulose and hemicellulose). Determination of cell wall compostition utilizing the detergent system is routinely performed in this laboratory and provides the basic information needed to determine forage quality as well as predict animal performance.

In vitro Digestion. In vitro fermentation represents the most reliable and accurate laboratory assay to estimate the portion of forage dry matter that is not degraded by rumen microorganisms. In vitro dry matter disappearance (IVDMD) is routinely determined by incubating forage samples in buffered rumen fluid for 48 to 72 hours. Residues recovered after incubation represent that fraction of the feed that is indigestible in the ruminant system. Digestibility is calculated as that portion of dry matter that disappeared or was not recovered after incubation. IVDMD is highly correlated to in vivo digestibility and allows rapid determination of digestibility for a relatively large number of forage samples at one time. In addition to IVDMD, kinetics of fiber digestion are also determined in this laboratory. Forage samples are incubated in buffered rumen fluid for 6, 12, 18, 24, 36, 48, 60, and 72 hours. This allows the relative rate of fiber digestion to be calculated as well as the digestion lag time.

Gas Chromatographic Analysis. One of the major research efforts in this

laboratory is the development of procedures for cell wall analysis utilizing gas chromatography (GC). Gas chromatographic analysis allows a much more detailed look at cell wall composition and provides a powerful tool to aid in our efforts to understand the relationship between fiber composition and structure and their effects on ruminal degredation. Recently, we have developed a new procedure for the separation and quantification of lignin derived alkaline labile phenolic monomers utilizing high resolution (capillary) GC. Quantification of these monomers is of interest as they have been shown to be negatively correlated to fiber digestibility. In addition to this work, we are also currently working on the adaption of procedures to separate and quantify structural carbohydrates utilizing high resolution GC.

**Near Infrared Reflectance Spectroscopy (NIRS)**

In this laboratory, NIRS is primarily used for routine determination of forage quality parameters, such as digestible dry matter, protein and fiber constituents. This technology has been used to estimate the quality of 1) leaf and stem tissue of insect-damaged alfalfa, 2) leaf, stem, and sheath tissue of normal and brown midrib sorghum x sudangrass hybrids, and 3) chemically regulated tall fescue and alfalfa-grass mixtures.

In a non-routine capacity, this laboratory is using NIRS for quantification of mold in alfalfa hay and endophytic mycelium in tall fescue. The NIRS procedure for alfalfa hay mold could introduce practical electronic hay marketing; the NIRS procedure for endophytic mycelium will be used to estimate true infection level of the fungal endophyte, *Acremonium coenophialum*.

In a cooperative capacity, the spectrophotometer has been used to analyze tissue of white-tailed deer diets, such as wild carrot, greenbriar, lanceleaf, poison ivy, japaneese honeysuckle, persimmon, dogwood, sugar maple, oak, hickory, and other wild plant species. This technology has also been used to quantify water, fat, and protein in bovine organ tissue.

# 1987 NCR-138 (Alfalfa Diseases) Report

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## 1. PROTOPLAST AND CHLOROPLAST DNA RESEARCH (LBJ, MRT)

### a) Protoplasts

We have shortened the regeneration time for plants from alfalfa protoplasts through modifications of the media. Somatic embryo formation is now observed within a month after plating. This should enhance the chances of producing somatic hybrids with our material. Efficient electrofusion-induced and polyethylene glycol-induced fusions are now possible between protoplasts of alfalfa and other Medicago species such as M. scutellata, and heterokaryons have divided. Since selection schemes for heterokaryons are available, we anticipate that somatic hybrid calli, if not plants, should be forthcoming. Several of the fusion partners for alfalfa have been selected for their disease and insect resistance traits. During efforts to develop selection schemes, alfalfa plants transformed with a gene for kanamycin resistance have been obtained, and other transformations are in progress.

### b) Chloroplast DNA

Crosses have been made to permit confirmation by chloroplast DNA restriction patterns of purported biparental inheritance in alfalfa. Studies of existing populations of various Medicago species have revealed single plants heterogeneous for chloroplast restriction patterns, a likely result of pollen transmission. In additional cooperative research with Dr. J. D. Palmer (Univ. of Michigan), phylogenetic relationships within Medicago are being determined by chloroplast DNA restriction mapping. The genus exhibits an unexpectedly high frequency of chloroplast DNA rearrangements.

## 2. DOWNY MILDEW

### a) Genetics of resistance in diploid alfalfa (DZS)

Previous work indicated that major and minor genes in alfalfa controlled resistance to Peronospora trifoliorum and suggested that the effects of minor genes were general with respect to the three pathogenically different P. trifoliorum isolates used. General resistance is very useful as it is not overcome by new genotypes of the fungus. However, results of further cycles of selection for resistance within populations without the major genes and

backcross data both indicated that the apparent general resistance was at least partially, and likely entirely, due to a collection of genes with isolate-specific effects.

b) Etiology and epidemiology (DMS)

Scanning electron microscopy revealed that P. trifoliorum germ tubes form appressoria between ridges on the anticlinal walls of epidermal cells of alfalfa leaves. Rabbit antiserum to P. trifoliorum conidia was successfully produced and utilized with fluorescent antibody techniques to detect P. trifoliorum hyphae and haustoria in alfalfa crown tissue.

c) Evaluation of resistance in Plant Introduction accessions (DLS, DZS, DMS)

We are continuing to cooperate with the North Central Regional Plant Introduction Station at Ames, Iowa, in the evaluation of PI accessions for resistance to isolates I-7 and I-8 of P. trifoliorum. To date, over 700 accessions have been evaluated. The results are available in the USDA Germplasm Resources Information Network (GRIN) computer system.

3. RUST (DZS, DLS)

We determined the effects of temperature on inheritance of resistance in alfalfa to the rust fungus, Uromyces striatus, using a six-parent diallel cross of diploid alfalfas. The percent of plants resistant (free of pustules) was 63, 50, and 70, and the degree of dominance of resistance was 0.59, 0.32, and 0.98 at 20, 25, and 30°C, respectively. Temperature significantly altered the genetic behavior of factors involved in alfalfa resistance to rust.

4. VERTICILLIUM WILT (WGW, TS, DLS)

Verticillium albo-atrum was first identified on alfalfa in Kansas in May, 1985, in southwestern Kansas. In 1985, it was found in eight irrigated fields in three contiguous counties. Since then, extensive surveys throughout Kansas have revealed it in only one additional, adjacent field. In May, 1987, infected plants were apparent in fields identified in 1985 but the disease is progressing slowly.

5. DEVELOPMENT OF MULTI-PEST RESISTANT ALFALFA GERMPLASM (DLS)

Alfalfa germplasms resistant to diseases and insect pests important in the Great Plains are being developed in cooperation with E. L. Sorensen, USDA and Department of Agronomy, and E. K. Horber, Department of Entomology. Tandem and independent culling procedures are used in conjunction with phenotypic recurrent selection. Two germplasms with multiple pest resistance were released during the past year. KS94GH6 was derived from diploid Medicago glandulosa subjected to six cycles of selection to increase erect glandular-hair density. It contains plants with high resistance to weevil, potato leafhopper, blue alfalfa aphid, spotted alfalfa aphid, anthracnose, downy mildew, rust, and summer black stem. KS189, derived from Sirsa #9, was selected for resistance to anthracnose (4 cycles), bacterial wilt (2 cycles), downy mildew (8 cycles), Fusarium wilt (2 cycles), Phytophthora root rot (6 cycles), blue aphid (2 cycles), pea aphid (6 cycles), and spotted alfalfa aphid (6 cycles).

J. A. Thies, D. K. Barnes, D. L. Rabas, and R. F. Nyvall
1987 NCR 138 Report
Departments of Plant Pathology and Agronomy and Plant Genetics
University of Minnesota and USDA-ARS
St. Paul, MN 55108
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Grand Rapids, MN 55744

In cooperation with the Department of Agronomy and Plant Genetics, alfalfa entries submitted by industry, public institutions, and the Minnesota alfalfa project, were evaluated for resistance to bacterial wilt (321 entries), Fusarium wilt (319 entries), and Phytophthora root rot (144 entries) in field tests at St. Paul and Rosemount, Minnesota. One hundred seventy-five of the entries in the bacterial wilt and Fusarium wilt nurseries were plant introductions (P.I.) being evaluated as part of a national P.I. evaluation program.

Studies were planted in June 1987 in cooperation with the Department of Agronomy and Plant Genetics and the North Central Experiment Station at Grand Rapids, MN. Field evaluation and selection tests for root-lesion nematode resistance were offered to commercial seed companies (on a fee basis) and public institutions. They are the first tests available for root-lesion nematode resistance. The tests are being conducted in three locations where very large numbers of root-lesion nematodes are present. Nematode populations at Location 1 (present University of Minnesota Experiment Station, Grand Rapids) are predominantly root-lesion nematodes (Pratylenchus penetrans) of about 500 nematodes/100cc soil. Location 2 (land recently purchased by the University of Minnesota Experiment Station, Grand Rapids) has about 350 root-lesion nematodes and about 1200 pin nematodes (Paratylenchus spp.)/100cc soil. Location 3 (near Bemidji, MN) has about 1000 lesion nematodes and about 100 spiral nematodes (Helicotylenchus spp.)/100cc soil.

The plots were established with carbofuran, which will control the nematodes for 4-6 weeks. This allows good stand establishment and makes weed control easier. Our previous research indicates that the frequency of resistant plants selected from carbofuran treated and untreated plots will be similar 2 years after establishment. It is planned that the plots will be harvested twice during 1987 and three times in 1988. Disease and general appearance notes will be taken in both years. In 1988, the third harvest will be made by September 1 and the plots will be undercut during the third week in September. Individual plant selection based on healthy top, crown and root characteristics, and numerous fibrous roots will be made by the originating company or experiment station scientists at this time. These selection techniques have produced experimental lines that have performed well at Grand Rapids and at several other locations in the Midwest.

A policy prohibiting seed treatment of alfalfa entries evaluated in the north central states in yield, disease, and pest trials was established at the Twenty-ninth Alfalfa Improvement Conference at Lethbridge, Alberta, Canada. We developed a petri dish test which allows detection

of seed treated with metalaxyl. A 1 cm diameter plug of actively growing Phytophthora megasperma drechs. f. sp. medicaginis was placed in the center of a plate of V-8 medium and allowed to grow at 25C for 3-4 days. Seeds were placed in a triangular pattern about 1 cm outside the fungal plug. This included 10 seeds per side each of: a check entry treated with metalaxyl, the same check entry untreated, and the entry to be evaluated. After 3-4 days the tests were evaluated. The fungus grew over the untreated seeds and prevented germination of most seeds. When seed was treated with metalaxyl, the fungus grew up to but not over the seed, and the seed germinated and was quite healthy. All alfalfa germplasm entered in Minnesota evaluation tests in 1987 were evaluated in this manner. A test substituting Pythium spp. for P. megasperma yielded similar results. We will continue to evaluate seed for metalaxyl treatment in subsequent years.

NCR-138 REPORT

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

Phytophthora/Aphanomyces

We have continued investigations on Aphanomyces euteiches as a pathogen of alfalfa alone and in combination with Phytophthora megasperma f. sp. medicaginis. The impact of A. euteiches on alfalfa is not known, but its frequent association with P. megasperma f. sp. medicaginis leads us to speculate that both pathogens act together in the form of a disease complex. In many areas of Wisconsin Phytophthora-resistant cultivars establish and persist longer than susceptible cultivars in wet-soil environments, but still decline at an unsatisfactory rate when both P. megasperma f. sp. medicaginis and A. euteiches are present.

We have detected low levels of resistance to A. euteiches in commercial alfalfa cultivars and have increased resistance by recurrent phenotypic selection. Populations have been improved for resistance to A. euteiches by selection against the pathogen alone in a controlled environment or in naturally infested field sites. Such populations have shown a yield advantage over nonselected populations when compared in plots naturally infested with A. euteiches and P. megasperma (Table 1). However, resistance to A. euteiches must be combined with resistance to P. megasperma to achieve such results.

Table 1. Comparison of forage yield of alfalfa populations selected or nonselected for resistance to Aphanomyces euteiches

| | % Res. Plants | | Total Forage Yield ($g/m^2$) | |
|---|---|---|---|---|
| Line | Aph. | Phy. | Site 1 | Site 2 |
| Aph-2 | 30 | 69 | 1073 | 909 |
| Aph-3 | 31 | 45 | 1062 | 940 |
| Aph-12 | 44 | 56 | 1037 | 859 |
| Armor | 2 | 41 | 1002 | 754 |
| Apollo II | 2 | 40 | 980 | 742 |
| Trident | 1 | 71 | 945 | 723 |
| Iroquois | 5 | 1 | 920 | 736 |

Aph-2 field selection from Apollo II; Aph-12 greenhouse selection (2 cycles) from Apollo II; Aph-3 field selection from Armor. Lines were evaluated in two plots naturally infested with A. euteiches and P. megasperma at the Marshfield Research Station. Forage yields are a total for three harvests in 1986. Plots were seeded in 1985.

Alfalfa plants were selected from the cultivars Vernal and Apollo II and, based on progeny reactions of individual plants, were placed into four classes; no resistance, resistance to Aphanomyces, resistance to Phytophthora, and resistance to both pathogens. Cuttings were made from individual plants within each group and were transplanted at two field sites; one site was naturally infested with both pathogens and a second site where both pathogens were not detected by baiting techniques. Forage yield of individual plants were recorded for three harvests in 1986. Plants that were resistant to both pathogens produced 14% less forage in the infested site.

However, plants with no resistance or resistance to only one of the two pathogens produced 64 to 86% less forage at the infested site when compared to forage production at the noninfested site.

Currently we are investigating the relationship between resistance to P. megasperma and A. euteiches. One question is whether selection for resistance against one pathogen affects the level of resistance to the other in the same alfalfa population. The populations Apollo II, Aph 1 and Aph 12 were independently inoculated with A. euteiches and P. megasperma, evaluated and 50 class 1 and 2 (1-5 severity scale) plants were selected from each population and polycrossed to produce a new population. A modified Apollo II population was developed by selecting 50 uninoculated, vigorous plants which also were polycrossed. Progeny of selected and nonselected plants were inoculated with A. euteiches and were evaluated for disease severity. Selection for Phytophthora resistance did not increase the level of resistance to Aphanomyces. In addition, one cycle of selection for Phytophthora resistance did not drastically alter the level of resistance to Aphanomyces in populations previously selected for resistance to the latter pathogen (Table 2). Progeny of all populations currently are being evaluated for resistance to Phytophthora.

Table 2. Changes in alfalfa populations for resistance to Aphanomyces euteiches after selection for resistance to A. euteiches or Phytophthora megasperma, or no selection against either pathogen

| Population | Selection | Plants per Severity Class 1 | 2 | 3 | 4 | 5 | ASI |
|---|---|---|---|---|---|---|---|
| Apollo II | nonpath | 0 | 0 | 16 | 61 | 23 | 4.06 |
| Apollo II | none | 0 | 1 | 18 | 59 | 23 | 4.03 |
| Aph 1 | none | 13 | 25 | 23 | 17 | 22 | 3.12 |
| Aph 12 | none | 22 | 22 | 34 | 13 | 9 | 2.64 |
| Apollo II | Aphan | 4 | 26 | 37 | 23 | 10 | 3.09 |
| Apollo II | Phyto | 0 | 0 | 24 | 55 | 21 | 3.97 |
| Aph 1 | Aphan | 20 | 32 | 30 | 9 | 9 | 2.56 |
| Aph 1 | Phyto | 7 | 22 | 37 | 27 | 7 | 3.06 |
| Aph 12 | Aphan | 28 | 27 | 28 | 12 | 5 | 2.48 |
| Aph 12 | Phyto | 20 | 23 | 35 | 7 | 15 | 2.77 |

Sclerotinia Stem Rot

Sclerotinia stem rot was quite prevalent at the University of Wisconsin Experiment Station located at Marshfield in October, 1986. Symptomatic and nonsymptomatic plants were marked in the fall and inspected for recovery in April, 1987. All nonsymptomatic plants showed stem growth in the spring. However, 50% of the symptomatic plants showed no regrowth and the remaining plants were weak in recovery. Most diseased stems expressed typical lesions well above the crown suggesting that ascospores were a major source of inoculum in the fall. Wilting stems with and without basal stem lesions were typical the following spring. Inspection of crown tissues revealed extensive crown decay and the presence of sclerotia. Cultivars and experimental lines in a yield evaluation trial established in 1984 were evaluated for their reaction to Sclerotinia stem

rot in October, 1986. Most cultivars or lines expressed a similar mean severity, but several consistently expressed high or low severity (Table 3).

Table 3. Disease reactions of alfalfa varieties to Sclerotinia stem rot at the Marshfield Experiment Station, October 1986

| Entry | DSI | Entry | DSI | Entry | DSI |
|---|---|---|---|---|---|
| Apollo | 3.33 | K5050 | 2.67 | Preserve | 2.33 |
| DS 307 | 3.33 | Advantage | 2.67 | Trumpetor | 2.00 |
| AS 67 | 3.33 | NAPB 20 | 2.67 | 120 | 2.00 |
| ICO 2[B] | 3.33 | 448 | 2.67 | Iroquois | 2.00 |
| H 134 | 3.33 | Trident | 2.67 | Saranac AR | 2.00 |
| Drummor | 3.33 | Polar II | 2.67 | CA 744 | 2.00 |
| G2815 | 3.33 | ICO 16[B] | 2.67 | Hiphy | 2.00 |
| Decathlon | 3.00 | Armor | 2.67 | Maxim | 2.00 |
| Peak | 3.00 | Magnum | 2.67 | WL 316 | 2.00 |
| LL3018 | 3.00 | Citation | 2.33 | C8-1 | 2.00 |
| WL 315 | 3.00 | NAPB 21 | 2.33 | 82-5 | 2.00 |
| 80335 | 3.00 | Apollo II | 2.33 | Oneida | 2.00 |
| 526 | 3.00 | Challenger | 2.33 | Saranac | 2.00 |
| Shenandoah | 3.00 | WL 221 | 2.33 | Futura | 2.00 |
| AS 60F | 3.00 | 532 | 2.33 | 83589 | 2.00 |
| DS 308 | 3.00 | Convert | 2.33 | 447 | 2.00 |
| 95V | 3.00 | Spectrum | 2.33 | NAPB 23 | 2.00 |
| LL3110A | 3.00 | Endure | 2.33 | NY 8301 | 2.00 |
| Blazer | 3.00 | Answer | 2.33 | Mohawk | 2.00 |
| NY 8302 | 2.67 | Chippewa | 2.33 | ICB-31 | 2.00 |
| Epic | 2.67 | W8240 | 2.33 | K7070 | 2.00 |
| WAI | 2.67 | G7730 | 2.33 | WARPS | 2.00 |
| Agate | 2.67 | Cimarron | 2.33 | W83-49 | 2.00 |
| NAPB 22 | 2.67 | WL 313 | 2.33 | | |
| Classic | 2.67 | WL 320 | 2.33 | | |
| Thunder | 2.67 | Olds 88 | 2.33 | | |
| 90R | 2.67 | Vernal | 2.33 | | |

LSD (0.05) = 0.85
CV = 21%

Disease Severity Index; 1 = no symptoms, 2 = low incidence (<5%), 3 = moderate incidence (>5% & <15%), 4 = High incidence (>15% & <25%). Values are the means of three replications.

# MINUTES OF THE 1987 CENTRAL ALFALFA IMPROVEMENT CONFERENCE BUSINESS MEETING

Chairman John Caddel called the meeting to order at 1:00 p.m. on June 24, 1987. The minutes of the 1986 business meeting were read and approved.

The status of the North American Alfalfa Improvement Conference subcommittee on the standardization of anthracnose resistance evaluation was asked by Don Barnes. This committee consists of Craig Grau, John Caddel, Stan Ostazeski, Wayne Hartman and Jim Baumer. Chairman Grau indicated that a report by the committee will appear in the Proceedings of the 1986 NAAIC. Don Barnes stressed the importance of research to better understand the apparent race specific and nonrace specific resistance that functions in alfalfa.

John Caddel indicated that he will remain responsible for the CAIC Alfalfa Variety Yield Evaluation Summary during his stay in Tunisia.

Edgar Sorensen lead a discussion on the topic of check cultivars for standard tests for yield, pest resistance and dormancy. After considerable discussion, Edgar Sorensen made a motion that a CAIC Standard Test Committee be formed and that it strive to solve some of the problems associated with maintaining standard cultivars, selection of new standard cultivars, report to the CAIC each business meeting and that it functions as a CAIC representative to the NAAIC Standard Test Committee. The motion was seconded by Mark McCaslin and passed. Chairman Caddel appointed Tim Woodward as chairman of the CAIC Standard Test Committee and was asked to select additional members of the committee. It was suggested that the CAIC committee contact the chairman of the NAAIC committee on Standard Test Committee and suggest names of CAIC members that could serve on the NAAIC committee.

Ray Clark indicated that the alfalfa plant introduction collection will move from Ames, Iowa to the Plant Introduction Station at Prosser, Washington at the end of August.

USDA funds are soon to be available for the improvement and utilization of alfalfa germplasm. Don Barnes solicited ideas and plans from the CAIC membership for use in drafting a national proposal to secure funds for alfalfa.

Mike Tesar indicated that Jim Elgin would be sending out forms for nomination of people to be recognized for major contributions to the alfalfa industry. Mike Tesar urged the CAIC membership to consider people worthy of this honor that are located in the Central Region.

Resolutions Committee Report

Be it resolved that the participants of the 20th Central Alfalfa Improvement Conference, held at the Holiday Inn, Urbana, Illinois, June 23-24 and the University of Illinois, would hereby like to express our sincere thanks and appreciation to the following:

John Caddel, Conference Chairman, for his many efforts in behalf of the Conference the last two years.

Richard Berberet, Program Chairman for the 20th Conference, for an excellent job of assembling an educational and stimulating program on the breeding, management and protection of alfalfa.

Darrell Miller, Local Arrangements Committee Chairman, and committee members, Don Graffis, Ken Moore, Walker Kirby, Ed Jaster and Kevin Steffey, for arranging fine meeting and rooming accommodations, excellent meals and outstanding tours of the forage and dairy research facilities.

Walker Kirby, Chairman, NCR-138 committee on alfalfa diseases, for organizing an excellent forum to discuss the latest findings on alfalfa pathology and the willingness to open the meeting to all Conference participants.

The management of the Holiday Inn, for fine meeting and sleeping room accommodations and the preparation of excellent meals.

Resolutions Committee,

John Caddel
Craig Grau

Locations Committee

The University of Wisconsin extends an invitation to hold the 21st Central Alfalfa Improvement Conference in Madison during the third week of June, 1989.

Location Committee,

Craig Grau

The motion was made, seconded and passed.

Nomination Committee

Mark McCaslin moved that Tim Woodward be nominated for Secretary. A motion that nominations cease was made by Don Stuteville. Both motions were seconded and passed.

Nomination Committee,

Mark McCaslin

The meeting was adjourned at 2:00 p.m.

Respectfully submitted,

Craig R. Grau, Secretary

**20TH CENTRAL ALFALFA IMPROVEMENT CONFERENCE**
Welcome to the University of Illinois
Departments of Agronomy
Plant Pathology and Agricultural Entomology

June 22 - 25, 1987

We are grateful to the following companies for helping sponsor the barbecue lunch and various breaks at the Conference. Please help us express your thanks to the following:

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Tour on Tuesday afternoon:

**Agronomy - Plant Pathology South Farm**
Welcome - South Farm
Alfalfa Cultivar Evaluation and Establishment - D. W. Graffis
Alfalfa Breeding - D. A. Miller
Alfalfa Disease Research - H. W. Kirby
Alfalfa Insect Researching - K. L. Steffey

**Animal Sciences - Dairy Science**

Dairy Automation - Hoyle Puckett
Automated Feeding - Ken Moore
Heifer Facility - Craig Roberts

Tour on Wednesday afternoon:

**Turner Hall - Forage Quality Laboratory**

Forage Cell Wall Analysis - John Fritz
Mold Detection Using NIR - Craig Roberts
Automated Data Collection - Robert Gerhart

CENTRAL ALFALFA IMPROVEMENT CONFERENCE - 1987
UNIVERSITY OF ILLINOIS AT HOLIDAY INN, CHAMPAIGN, IL
REGISTRATION

| | Name: Last | First | MI | Organization | Address | City | St | Zip |
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