

**Biomass Energy Production Alfalfa Supply System**

# **Alfalfa Variety Development**

**MINNESOTA AGRIPOWER PROJECT  
TASK II RESEARCH REPORT**

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## **ALFALFA VARIETY DEVELOPMENT FOR USE AS BIOMASS TO PRODUCE ELECTRICITY**

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### **Objective:**

Produce specialized alfalfa varieties specifically adapted to the unique demands of a biomass production system to produce electricity and high quality leaf meal.

### **Introduction:**

The drawback of many biomass fuels ( wood chips, sawdust, and residues from food processing) have been the proximity to the power plant and the long-term availability of the fuel supply. A dedicated feed stock supply system would guarantee a consistent long-term fuel supply. Alfalfa was chosen as the dedicated feed stock resource and is proposed to be managed under a two harvest schedule to maximize stem yield, enhance wildlife habitat, and minimize production costs. The hay will be fractionated into stem material for conversion to electricity and leaf meal to be sold as a livestock protein supplement. An alfalfa biomass variety adapted to the upper midwest would include the following traits: winter hardiness, resistance to major pathogens, resistance to foliar disease complexes, many thick, tall, solid, non-lodging stems with high lignin content, delayed flowering, and high quality leaves retained through harvest. Currently no alfalfa varieties meet these criteria.

### **Breeding Methodology:**

Modern commercial alfalfa varieties have been selected for use as feed for ruminant animals. Plant breeding efforts and best management practices for increased forage quality have led to increased leaf to stem ratio because leaves are much more digestible and higher in protein than stems. However, in biomass production systems, lignified plant materials such as stems would be the component of interest to maximize energy output. To meet the economic demands of this new biomass production system the yield of both leaf and stem fractions will need to be maximized. Development of a biomass variety requires that plant breeders use older European germplasm sources that will provide the needed stem traits and resistance to some foliar diseases but these germplasm sources lack winterhardiness and are susceptible to many root and crown diseases. Genetic sources for winterhardiness and resistance to major pathogens are available and can be introgressed into selections for biomass by crossing these experimental populations to modern adapted and disease resistant cultivars.

### **Current Experimental Germplasms:**

#### **1. Biomass I (UMN 3064)**

This alfalfa population was created by crossing ORCA-WTS [created by selecting for large stems in an old central European (Flemish type) variety named ORCA, which is taller and has a greater stem yield compared to modern varieties, is known to have larger stems and some lodging and foliar disease resistance, is moderately dormant (rating=5) and is susceptible to winter kill and diseases that are prevalent in Minnesota] to other more modern Flemish alfalfa sources. After two cycles of selection for large stems and winter survival it is about 75%

Flemish. It is very tall and has large stems, but is susceptible to several diseases and is moderately dormant.

2. Biomass II (UMN 3065)

This population was created by crossing ORCA-WTS (see description above) to dormant alfalfa populations. After two cycles of selection it is approximately 50% Flemish. Stems are larger than modern alfalfa varieties but smaller than those found in Biomass I.

3. ORCA-WTSxMWNC-HF<sub>C2</sub>-BRH (UMN 2995)

This population is a strain cross between ORCA-WTS and modern dormant, multiple pest resistant experimental population selected for increased fibrous root mass and number of branched roots.

**Current Progress:**

All three experimental biomass populations have been evaluated for resistance to economically damaging diseases including: Spring Blackstem, Phytophthora root rot, Verticillium wilt, Bacterial wilt, Fusarium wilt, and Aphanomyces root rot (Table 1.) All populations have some resistance to these diseases but increasing resistance to these pathogens will be critical to maximize leaf and stem yield in these biomass populations.

A third cycle of selection for large, non-lodging stems and winter survival for the experimental biomass populations has been completed. Selected plants were also screened for minimal incidence of foliar disease and have been moved to the greenhouse for crossing.

Retention of leaves through delay of leaf senescence and resistance to foliar diseases will be of major importance. Cytokinins, a type of plant growth regulator, delays senescence in alfalfa when applied to leaves in labeling tests. Inhibition of leaf senescence was reported in tobacco using a chimeric gene composed of an *Arabidopsis* senescence-specific gene promoter (SAG12) and an *Agrobacterium* gene encoding isopentenyl transferase (IPT), the enzyme that catalyzes the rate-limiting step in cytokinin biosynthesis. This Chimeric gene was introduced in alfalfa and transgenic plants were produced. The SAG12 promoter was not active in alfalfa leaves. Isolation and characterization of alfalfa senescence-specific genes is currently underway in order to identify an alfalfa promoter for expression of the IPT gene.

It is expected, based on published results from tobacco, that expression of the transgene will increase dry matter yield by up to 50% because of leaf retention and prolonged photosynthetic life-span of leaves. Inhibition of senescence should also increase disease resistance to a number of foliar pathogens.

**Remaining work to produce competitive alfalfa biomass varieties:**

The experimental biomass populations need to be crossed to produce seed for the latest cycle of selection. All populations will then need to be selected for increased resistance to Phytophthora root rot, Bacterial wilt and foliar leaf diseases to produce successful varieties under field conditions. When the senescence inhibition is obtained in alfalfa, the trait will be crossed into the best populations selected for biomass production and the progeny evaluated. Disease resistant biomass populations will then need undergo seed increase to have enough seed available for commercial release.

Table 1. Biomass Entries Disease Evaluation Summary, Seedling Greenhouse Tests 1996-1997.  
Conducted by D.A. Samac

Entry	Blackstem		Phytophthora Root Rot		Verticillium Wilt		Bacterial Wilt		Fusarium Wilt		Aphanomyces Root Rot	
% Resistant*												
UM 3064 Biomass I	29	8			9			14		22		20
UM 3065 Biomass II	26	7			10			26		46		20
UM 2995 MWNC-HF <sub>c2</sub> BRH x ORCA	24	9			8			19		45		19
Checks:												
Resistant	12	29			26			39		44		44
Susceptible	5	1			5			1		5		1

\* 0-5% Susceptible (S)  
6-14% Low resistance (LR)  
15-30% Moderate resistance (MR)  
31-50% Resistance (R)  
<50% High resistance (HR)