ALFALFA (*MEDICAGO SATIVA* L.) SCREENING FOR POST-HARVEST FUNGAL RESISTANCE

V.I Babij[1], K. M. Wittenberg[1], and S.R. Smith, Jr.[2]

[1] Department of Animal Science

[2] Department of Plant Science University of Manitoba, Winnipeg, Manitoba R3T 2N2

ABSTRACT

A leaf screening procedure developed by Wittenberg et al. (in preparation) for detection of plants resistant or susceptible to fungal growth after cutting was compared with fungal growth on whole plants were chopped and stored under warm humid conditions in the laboratory or wilted and baled in a simulated field trial. Four genotypes previously identified as having low, variable and high susceptibility to fungal growth after harvest were used. Extent of fungal growth, as measured by glucosamine analysis, for plant material chopped and incubated under conditions conducive to molding, supported plant resistance ratings as determined by the screening procedure. Minimal fungal growth across all genotypes in the simulated field wilting made comparative evaluation impossible. Mini bales incubated under simluated stack storage conditions for 9 d showed glucosamine differences between the resistant and susceptible genotypes supporting the screening methodology. A screening of 1144 genotypes representing 22 cultivars of alfalfa for post-harvest resistance to fungal growth demonstrated that there is variation for this trait.

KEYWORDS

Alfalfa, mold, glucosamine, fungal resistance, genotypes.

INTRODUCTION

Alfalfa cultivars have been developed for desirable qualities such as high leaf production, stem size, resistance to frost, and grazing tolerance, but no selection protocols have been developed for forage resistant to post-harvest fungal invasion. Fungal organisms, which include the genera *Mucor, Absidia, Rhizopus, Aspergillus* and *Humicola,* develop in inadequately dried hay and reduce forage quality. These organisms also produce spores which are a major cause of respiratory problems for livestock and livestock producers. An effective method for screening alfalfa for post-harvest fungi could be beneficial in developing cultivars with improved resistance to saprophytic organisms during field wilting, and potentially could allow harvest of hay at a higher moisture content. Baling hay at a higher moisture content reduces quality decline due to inclement weather and leaf loss.

A screening procedure to determine post-harvest resistance by challenging the freshly harvested plant leaf to five *Aspergillus spp.* was recently developed in this laboratory (Wittenberg et al, in preparation). The objectives were to determine how well the screening procedure compared to plant molding as determined by glucosamine analysis, a measure of fungal cell wall constituents. Plant response to the specific organisms used in the screening procedure was also compared. Finally, the screening procedure was applied to genotypes representing currently used and newly developed cultivars to determine the range in resistance to the trait for post-harvest resistance to fungal growth.

METHODS

A screening study, paralleled with whole plant incubation, was conducted on 4 genotypes previously identified as having either low, variable or high susceptibility to fungal growth. Leaf material from the 4 freshly harvested genotypes was plated on DG18 agar in duplicate for the screening study. Plated material was then inoculated with a spore suspension (10^6 spores /ml) of the five individual species of *Aspergillus, A.flavus, A.glaucus, A.repens, A.versicolor,* and *A. fumigatus*, as well as a combined inoculum at a rate of 100 ul to 300 ul/plate. The combined inoculum consisted of equal aliquots of each of 5 Aspergillus species. Visual assessment was completed on day 5 when 70% of the plates had a minimum of 20% of the leaves colonized with fungal biomass.

Plants representing the same 4 genotypes were harvested and chopped. Four g of chopped material from each genotype was placed into ten empty petri plates, and incubated at 25ºC. Duplicate plates for each genotype were removed from incubation and frozen on days 5, 8, and 11 of incubation. Plates and contents were freeze dried for dry matter and glucosamine (Wittenberg et al., 1989) determinations.

The same 4 genotypes were used for field wilting and bale storage. Plants were cut and piled to simulate a windrow for each genotype. Forage samples were taken 1 hour post cutting, and daily at 9:00 and 16:00 h until the moisture content was approximately 30%. Samples were collected in sterile sample bags, freeze dried for dry matter and glucosamine determination.

Forage from the wilting study was used to make up 12 mini bales per genotype with a density of 437 kg/m^3. The bales were tied together in a stack and insulated with a combination of mini bales and styrofoam to minimize heat and moisture loss. The bale temperatures were monitored during incubation to ensure that the temperatures corresponded to normal hay stack temperature changes (Undi, 1995). Four bales representing each genotype were core sampled, freeze dried, and analyzed for dry matter and glucosamine levels on day 0, 9 and 24 of incubation.

The screening method developed by Wittenberg et al. (in preparation), was used to select a resistant population and a susceptible population from an initial population of 1144 genotypes representing 22 cultivars. Using the screening method, leaves were randomly removed from the entire plant, and placed onto DG18 agar plates using aseptic technique. Approximately 300 ul of a spore suspension at a concentration of 10^6 spores/ml was sprayed onto each plate. The plates were incubated at 25ºC, and the plates were evaluated when 70% of all the plates had 20% fungal coverage on the leaf material.

RESULTS AND DISCUSSION

Plant susceptibility to fungal attack did vary for the species of Aspergillus (Figure 1). The greatest spread between the low and highly susceptible genotypes was observed for *A. repens* and *A. versicolor*. In each case, the resistant genotype had the lowest percentage of leaf area covered by fungal biomass. Glucosamine concentrations, a measure of fungal cell wall, increased most rapidly for the susceptible genotype when whole plant material was incubated (Table 1).

Genotypes identified as susceptible and variable 2 had a more rapid rate of moisture loss than the other 2 genotypes during the field wilting trial. Glucosamine accumulation during field wilting was low

and difference could not be determined. Screening procedure verification also is supported by the bale storage trial as glucosamine concentrations of 3.82 and 2.47 mg/g on day 9 were significantly higher for the susceptible than the resistant genotype, respectively. The results of the screening showed that there were cultivar differences and genotype differences within cultivars. Cultivar Rambler was more susceptible (P<.05) to fungal growth after harvest than all other cultivars tested with the exception of Class and Rushmore. Cultivar Arrow was rated most resistant to fungal growth and was significantly more resistant than Rambler, Class, Rushmore, Algonquin, GH 787, Apollo Sup. and Pickseed 8920 MF.

Table 1
Mean glucosamine values (mg/g DM) of leaf and stem material before and during an 11 day incubation.

	Incubation Time			
Genotype	Day 0	Day 5	Day 8	Day 11
Resistant	1.18	2.17[a]	6.62	9.79[a]
Variable 1	1.34	2.15[a]	5.87	9.77[a]
Variable 2	1.25	2.85[ab]	6.06	10.40[ab]
Susceptible	1.24	3.05[b]	7.56	12.95[b]
SEM	0.20	0.20	0.82	0.60

[a, b] Means in the same column having different letters are different, P < 0.05 using Bonferroni test.

REFERENCES

Wittenberg, K.. R. Smith and F. Katepa Mupondwa. Screening Methodology for Post-Harvest Fungal Resistance in Alfalfa. In preparation.

Wittenberg, K.M., S.A. Moshtaghi-Nia, P.A. Mills and R.G. Platford. 1989. Chitin analysis of hay as a means of detecting fungal invasion during storage. Animal Feed Science and Technology **27**:101-110.

Undi, M. and K.M. Wittenberg. 1996. Effect of dry matter content at baling on charge in forage constitients during storage of alfalfa hay. Can. J. Anim. Sci. **76**: 599-605.

Figure 1
Extent of leaf area colonized when genotypes of alfalfa are incubated with one of five Aspergillus species or a combination of all five Aspergillus species.