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## Breeding forage legumes to complement warm season perennial grass pastures in the US southern region

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#### Introduction

Perennial warm-season grass pastures, primarily bermudagrass (Cynodon dactylon [L] Pers.) and bahia grass (Paspalum notatum Flugge), cover approximately 12 million hectares in the US southern region (US Census of Agric. 2002). These grasses are used for grazing, hay production or both. Bermuda grass and bahia grass are dormant from late fall until early spring with some variation in total dormancy period depending on seasonal conditions and latitude. Cool-season forage legumes can be over-seeded in the fall before the perennial grasses become dormant, providing winter grazing and nitrogen for the pasture system. The legumes sown in these grasslands are often acid tolerant species from the Trifolium genus as the predominant soils of the US southern region are sandy, acidic and highly leached. Legume breeding programs have been in place for 30 years with the general objective to develop more reliable forage legume cultivars to co-exist in these perennial grassland systems. Our improvement programs have addressed such problems as virus and fungal disease susceptibility (Pemberton et al. 1989; Pemberton et al. 1998) in arrowleaf clover (Trifolium vesiculosum Savi.), poor seedling regeneration (Evers and Smith, 2006) in crimson clover (T. incarnatum L.) and low persistence in white clover (T. repens L.). These research efforts are further described below.

#### Methods

Apache arrowleaf clover was developed through six cycles of selection for tolerance to bean yellow mosaic virus (BYMV) disease (Pemberton et al. 1994). Blackhawk arrowleaf clover was developed through three cycles of recurrent selection for tolerance to the fungal soil pathogen Pythium ultimum and one cycle of selection for tolerance to BYMV. Research is in progress to improve the reseeding ability of crimson clover through recurrent selection and progeny testing for a decrease in the rate of seed softening and a concurrent increase in seed survival (Smith et al. 2008). The experimental crimson cultivar TX-Late was developed through three cycles of recurrent selection for late flowering and two cycles of selection for high hard seed level. Fifty-five crimson clover half-sib breeding lines, TX-Late and two commercial cultivars were evaluated in a two year progeny test. Recurrent selection for early flowering in diverse white clover populations with multiple pest resistance was used to develop white clover

with improved adaptation to the US southern region. The diverse white clover populations included selections from the following: SRVR (Gibson *et al.* 1989); Brown Loam #6 (Knight *et al.* 1988) and MSNR4 (Pederson and Windham, 1995); PI 248985; PI 404930; and white clover ecotypes collected in Smith and Anderson counties, Texas, USA. These breeding programs were all conducted in concert with forage management and physiology research, using cow-calf grazing in one or more stages of development.

#### Results

#### Arrowleaf clover

Arrowleaf clover is very productive in the US southern region however older cultivars (Yuchi, Meechee, Amclo) have been very susceptible to aphid-transmitted virus diseases and to seedling fungal diseases. To address these cultivar deficiencies the Texas Agricultural Experiment Station released the cultivar Apache (Smith et al. 2004) and Plant Variety Protection status was granted in 2005. The BYMV tolerance of Apache, developed through multiple cycles of recurrent selection, has restored arrowleaf clover as a useful legume forage in livestock systems of the US southern region. Texas A&M AgriLife Research released Blackhawk arrowleaf clover in 2013 (GR Smith, pers. comm.). BlackHawk is highly tolerant of Pythium ultimum with 67% disease-free seedlings. In contrast Yuchi and Apache arrowleaf clover are highly susceptible to this fungal disease. Blackhawk is also resistant to BYMVinduced lethal wilt and shows tolerance to the other components of BYMV disease. Blackhawk seed are 95% black with only slight variation in degree of dark colouration. The black seedcoat has been associated with tolerance to Pythium ultimum in our experiments and further research is underway to determine the genetics of this seedcoat colour associated trait. If a successful relationship can be established, then seedcoat colour may be used as a phenotypic marker for improved resistance to Pythium ultimum in Arrowleaf clover.

#### Crimson clover

Self regeneration of crimson clover was developed in the 1950's (Hollowell 1953) however the older crimson varieties have declined in their reliability to regenerate. The crimson entries in this study varied widely in the percentage of seed that survived 320 days (320SS) for both test years. The range of 320SS was 1 to 24% and 1 to 47%

in 2006 and 2007 respectively. Five elite breeding lines were identified capable of producing progeny with 320SS greater than 22% in both evaluation years. In contrast the cultivars Dixie and Tibbee averaged 7.2 and 6.1% 320SS, respectively. The experimental cultivar TX-Late exhibited improved seed survival with a two-year average 320SS of 19.4%. Seed survival data from these experiments provides an indication of hard seed levels remaining in the soil seed bank after a winter, spring, and summer exposure to field conditions. This research indicated that variation exists within crimson clover to increase hard seed levels and hence improve soil seed bank persistence and seedling regeneration.

#### White clover

White clover is productive on clay and clay loam soils in bottomland sites (flats) but generally does not live through the summer in this region as a perennial due to numerous factors, including diseases, insect pests, nematodes, summer droughts and a shallow rooting depth (Pederson et al. 1991). Improvements in early flowering and seed production will allow white clover to regenerate from seed and persist in this region as a self regenerating annual in those years that it does not perenniate. To address this issue the cultivar Neches (Smith et al. 2008) was developed in the Forage Legume Breeding Program at Overton and released by Texas AgriLife Research in Jan. 2010. Neches is a synthetic variety of white clover with 147 parent plants selected for early and profuse flowering at Overton, TX in combination with moderate leaf size and high potential forage production. Neches combines early flowering and seed production with high forage yield and is broadly adapted on loamy bottomland soils from east Texas across the entire southern region.

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