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Considerations in Breeding Endophyte-Free Tall Fescue Forage Cultivars

J. F. Pedersen* and D. A. Sleper

Breeding tall fescue (*Festuca arundinacea* Schreb.) cultivars that are free of the endophytic fungus *Acremonium coenophialum* Morgan-Jones and Gams [previously identified and referred to as *Epichloe typhina* (Fries) Tulasne] is necessary to improve animal performance. The techniques used in developing new cultivars are not greatly different from those used previously, with one exception. Prior to the evaluation of new tall fescue lines or populations, the endophyte needs to be eliminated from the seed or the plants. Several techniques utilizing aging, heat, or chemical treatment are being used to effectively accomplish this in the seed. Methods for permanently eliminating the endophyte from plants are not available. The major new considerations in breeding endophyte-free tall fescue cultivars do not involve drastic changes in breeding methodology, but rather focus on new objectives. In the past, much effort was directed at overcoming the toxic effects of the endophyte. Now, breeders can focus their efforts on objectives

such as increasing digestibility, physiological efficiency, mineral uptake, and insect and disease resistance. Losses in stress tolerance due to the elimination of the endophyte from tall fescue may also have to be addressed, especially in areas of marginal adaptation.

Additional Index Words: *Acremonium coenophialum* Morgan-Jones and Gams, *Epichloe typhina* (Fries) Tulasne, *Festuca arundinacea* Schreb., Fescue toxicity, Plant breeding.

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Table 1. Forage tall fescue cultivars released in the USA prior to 1980.†

Cultivar	Year released	Selection criteria
Kentucky 31	1943	Good fall and winter growth
Alta	1945	Like above, but 5 days earlier in maturity
Goar	1946	High herbage yield, drought tolerance
Asheville	1952	Stable in adverse environments
Kenmont	1963	Good palatability, high crude protein, high herbage yield
Fawn	1964	High digestibility, high crude protein, high herbage yield, low self fertility
Kenwell	1965	Good palatability, foliar disease resistance
Kenhy	1977	Vigor, soft lax leaves, high forage moisture during drought stress
Missouri 96	1977	High animal performance, soft fine texture

† Adapted from Asay et al., 1979.

BASED ON the number of new cultivars entering the marketplace, current breeding programs for fungus-free tall fescue (*Festuca arundinacea* Schreb.) appear to be successful. What exactly is “fungus-free” tall fescue, and why has it affected forage breeding programs so dynamically? In popular usage “fungus-free” denotes tall fescue that is not infected with the endophytic fungus *Acremonium coenophialum* Morgan-Jones and Gams. Most tall fescue grown in the USA is infected with this endophytic fungus (Siegel et al., 1985), which causes poor performance in animals that consume it. Freeing tall fescue from this fungus has transformed it from a species that has been considered by some to be marginally useful to livestock producers, to a species with enormous potential as a broadly adapted, hardy, and high quality perennial cool season forage grass.

Several reviews concerning tall fescue have been published in recent years. An entire monograph on tall fescue was published by the American Society of Agronomy (Buckner and Bush, 1979). Tall fescue breeding was reviewed by Sleper (1985). The negative effects of *A. coenophialum* infected tall fescue on cattle performance are well documented (Hoveland et al., 1980; Hoveland et al., 1983; Pedersen et al., 1986; Siegel et al., 1985; Sleper, 1985; Read and Camp, 1986). This paper will concern itself with tall fescue breeding specifically as it is affected by our ability to deal with *A. coenophialum* infection in the grass.

Prior to the discovery of the endophyte in tall fescue (Bacon et al., 1977) and its association with poor cattle performance (Hoveland et al., 1980), tall fescue breeding objectives were predominately for improved forage quality and yield (Table 1, Asay et al., 1979). Intergeneric and interspecific hybridization was one method used in an attempt to overcome the poor forage quality thought to be inherent in tall fescue (Buckner, 1973; Buckner et al., 1961, 1967, 1976). However, the presence of the endophyte overshadowed the quality contributions made to tall fescue by introducing genes from such species as annual ryegrass (*Lolium multiflorum* Lam.).

Recently released forage tall fescue cultivars are being marketed as endophyte-free, but their selection criteria were similar to those released before 1980 (Table 2, Asay et al., 1979). In most cases, the endophyte-free status of

Table 2. Forage tall fescue cultivars released in the USA after 1980.†

Cultivar	Year released	Selection criteria
Forager	1980	High herbage yield, vigor, good persistence
AU Triumph	1981	High winter yield, open sod, erect growth
Johnstone	1983	Low peroline, improved succulence and digestibility during summer
Martin	1985	High seed and herbage yield, high magnesium content
Mozark	1985	High seed and herbage yield, good animal performance
Safe	1985	Vigorous seedlings and mature plants, pasture quality, drought tolerance, winter hardiness, and disease resistance

† Asay et al., 1979.

the new cultivars was produced accidentally, or by seed treatment. Therefore, the substantial improvements in animal performance on these new cultivars is probably more related to seed handling or seed treatment than to plant breeding because resistance to the endophyte has not yet been discovered.

WHAT DO BREEDERS GAIN?

In essence, they gain a “new” forage grass species. Tall fescue has adequate inherent quality for beef cattle production (Hoveland et al., 1980; Hoveland et al., 1983; Pedersen et al., 1986; Siegel et al., 1985; Sleper, 1985; Read and Camp, 1986). It is potentially a perennial replacement for annual grasses such as annual ryegrass and small grains in areas where no other perennial grass is adapted (Edwards and Pedersen, 1985). Elimination of the endophyte from tall fescue expands its potential uses to include dairy (Baxter et al., 1986) and horse (Wiggins et al., 1986) production, two industries not often using this species.

Most importantly, elimination of the endophyte from tall fescue allows plant breeders to develop programs with objectives other than overcoming the toxic effects of the fungus. These new objectives include increasing herbage yield, seed yield, digestibility, physiological efficiency, mineral uptake, and insect and disease resistance (Sleper, 1985).

WHAT DO BREEDERS LOSE?

It is becoming increasingly certain that some losses in performance may be associated with the elimination of the endophyte from tall fescue. This ultimately must be addressed through new breeding objectives. Insect resistance conferred to tall fescue by the presence of the endophyte has been observed for fall armyworm (*Spodoptera frugiperda* J.E. Smith) (Clay et al., 1985), Argentine stem weevil (*Listronotus bonariensis* Kuschell) (Barker et al., 1983), oat bird cherry aphid (*Rhopalosiphum padi* L.), greenbug (*Schizaphis graminum* Rondani), and large milkweed bug (*Oncopeltus fasciatus* Dallas) (Siegel et al., 1985). Fewer spiral nematodes (*Helicotylenchus* spp.) have been found under endophyte-infected tall fescue than endophyte-free tall fescue (Pedersen and Rodriguez-Kabana, 1986).

All the preceding results are from laboratory or greenhouse studies. The potential for increased insect or nematode susceptibility of endophyte-free tall fescue in the field has not been fully established. Both positive and negative associations between endophyte infection level in tall fescue and numbers of several insects have been observed in the field (Kirfman et al., 1986).

Improved persistence, fall recovery, and resistance to weed invasion in endophyte-infected tall fescue have been observed by Hurley et al. (1984) in old turf plots. Read and Camp (1986) demonstrated a lack of stand persistence in endophyte-free tall fescue, under grazing conditions in Texas. However, in other areas, no decline in stands of endophyte-free tall fescue has been observed (Pedersen et al., 1982). It is too early to predict what effects removing the endophyte from tall fescue will have on pasture stand persistence; but, it appears that negative effects will be more pronounced in marginal areas of adaptation, and will have to be addressed in breeding programs for those areas.

Breeders selecting for high yield, or improved physiological parameters, should also be aware that these traits may be influenced by the presence of endophytic fungi in some species. Latch et al. (1985) have shown enhanced growth in endophyte-infected perennial ryegrass (*Lolium perenne* L.). Read and Camp (1986) have shown higher forage production on endophyte-infected tall fescue pastures than on endophyte-free pastures. However, Siegel et al. (1984) reported tall fescue hay and seed yields to be independent of endophyte infection levels.

BREEDING ENDOPHYTE-FREE CULTIVARS

Source Nursery

The gathering of germplasm is an important first step in development of an endophyte-free tall fescue cultivar. A collection with a broad genetic base is desirable if significant progress is to be realized through selection. The plant materials collected are commonly space-planted into a source nursery. A source nursery may include 10 000 or more individual plants (Fig. 1). Materials for a source nursery may come from plant introduction stations around the world. Tall fescue is not native to the USA. It reportedly evolved both south and north of the Mediterranean Sea. It can be found in its native state in much of Europe and the Tunisian area of North Africa and in west and central Asia and Siberia (Borrill et al., 1971; Terrell, 1979). Other sources for inclusion into a source nursery are natural ecotypes or land races, germplasm pools created to produce unique gene combinations, old cultivars, and specific crosses among selected parents.

Eliminating the Endophyte

It is important to destroy the endophyte in seeds which are used to provide plants for inclusion into the source nursery if the objective is to produce an endophyte-free cultivar. If one is transferring infected vegetative material to the source nursery, it may be difficult to eliminate the



Fig. 1. Tall fescue source nursery.

fungus. Siegel et al. (1984) had limited success in removing the endophyte from potted tall fescue plants using bitertanol as a soil treatment. A better alternative is to destroy the viability of the fungus in the seed by long-term storage, short-term heat treatments, and seed applied fungicides. Siegel et al. (1984) found that viability of the endophyte was eliminated after 7 to 11 months of storage at 70 °F. Also, a heat treatment of 135 °F for 40 min or 120 °F for 7 d eliminated the endophyte. Of the chemical treatments evaluated on seed, certain triazole fungicides were the best in eliminating the endophyte using the criteria of cost, lack of significant phytotoxicity, and effectiveness of control. These treatments may lower germination percentage.

But all tall fescue breeders may not want to eliminate the endophyte from their plants. Turf breeders may want to develop tall fescue cultivars with the endophyte to confer levels of resistance to certain insect pests. In this instance, the source nursery should include plants that are highly infected with the endophyte. The endophyte can be added to desirable germplasm sources by outcrossing to plants containing the endophyte, using the infected plants as females, and harvesting seed from them since the endophyte is transferred by seed (Neil, 1941). It is also possible to artificially infect tall fescue plants with the endophyte (Latch and Christensen, 1985).

Phenotypic Selection

The forage grass breeder will want to incorporate desirable agronomic traits into new cultivars in addition to eliminating the endophyte. Agronomic traits of interest may include high herbage and seed yield, leafiness, optimal forage quality—such as high digestibility and lower cell wall contents, superior animal performance, increased tillering, persistence, and disease and insect resistance, as examples. Most of these traits are under the control of many genes.

The tall fescue breeder will evaluate the plants grown in the spaced-planted nursery for the traits under selection. If resources permit, the plant breeder should have the spaced-planted source nursery growing at more than one location to permit evaluation of clones in more than one environment. The breeder should observe plants for more than one year since tall fescue is a perennial and persistence of desirable growth habit is a goal of many tall fescue breeding programs. Natural selection over time may eliminate plants that lack adequate winterhardiness, insect and disease resistance, and drought tolerance. Selection of desirable plants from the source nursery is often based entirely on phenotypic performance.

Seeds are harvested from desirable maternal plants from the source nursery and may be combined to form a new population for further selection or for use in progeny testing.

Progeny Testing

After selection of desirable clones from the source nursery or selected population, it is necessary to deter-

mine which clones would combine well to form an experimental synthetic cultivar for further testing. This is accomplished by evaluating the progeny from selected clones. The topcross, polycross, and open-pollinated progeny tests are examples of procedures used to evaluate general combining ability of potential parents for making experimental synthetic cultivars (Poehlman, 1979). General combining ability is defined as the average performance of a strain in a series of crosses. After a progeny test is completed, the tall fescue breeder is provided data which will indicate the best clones to serve as parents for the new experimental synthetic cultivars. More than one experimental synthetic may be produced from a given germplasm source.

Experimental Synthetics

Seed production of the endophyte-free synthetic begins with the establishment of an isolated polycross nursery. Selected clones are replicated to insure random mating. Equal amounts of pure live seed are harvested from all replicates of each clone to obtain the first synthetic generation (syn-1) seed. This may also be used as breeder seed. The syn-1 seed is advanced to at least the second (syn-2) or third (syn-3) synthetic generation to have adequate seed stocks for testing in small plots or animal trials. Early generation synthetics may have a high degree of heterosis and the performance may be better than later generations. Seed purchased by growers will often be fourth synthetic generation (syn-4) or later

Preliminary Testing

Experimental endophyte-free synthetics are seeded along with check cultivars into small replicated plots. Plots are usually established at several locations and evaluated from 3 to 5 yr. The types of data collected from the small plots will depend on the objectives of the breeder. One should certainly monitor the population for the endophyte to insure against contamination. Other data collected might include flowering date, *in vitro* dry matter digestibility, herbage yield, and persistence.

Animal Performance Trials

The best performing endophyte-free synthetics from the small plot test, along with selected checks, will be used in this phase of cultivar development. The acid test of any endophyte-free cultivar is animal performance. Techniques used in animal evaluation of endophyte-free tall fescue experimental cultivars should be related to output per animal and output of animal product per acre. The number of experimental entries used in this phase may be limited (only 1 to 3) because of the expense and labor requirements of animal trials.

An example of a technique for using small pastures to evaluate animal response when grazing experimental endophyte-free cultivars has been discussed by Matches et al. (1981). Entries are seeded in one-acre pastures and replicated four times in a randomized complete block

design. Replication number 4 serves as a reserve pasture on which animals are conditioned before testing, and where animals are maintained between grazing periods. Cattle weighing between 450 and 550 lb at the start of the grazing period are selected. Grazing periods are from 35 to 68 d. In Missouri, separate periods are used in the spring, summer, and fall. Three animals generally graze each pasture in the spring and fall and only two graze each pasture in summer when less forage is available. Cattle are weighed (based on shrunk weight) every 2 wk following 16 h of confinement without feed or water.

Equal grazing pressure (same amount of feed per animal) is maintained by adjusting the area grazed with movable fences. Animals are allotted a daily amount of herbage on a dry weight basis equivalent to approximately 2.5% of their live weight and are moved to a fresh strip each week. Pasture strips are sampled weekly to estimate the amount of herbage available one day before grazing and the amount of residue remaining after grazing, with the difference approximating intake.

Table 3 contains animal performance data of several endophyte-free cultivars and experimental synthetics of tall fescue averaged over 4 yr. These data are the result of experiments conducted following the preceding suggested design for evaluating new experimental synthetics.

Because there is concern that endophyte-free cultivars may not be as persistent as infested ones, studies may be designed to evaluate the plant's response to the grazing animal. The experimental strains may be seeded in replicated plots and subjected to heavy grazing and trampling by animals. Data collected may include ability to recover after grazing and persistence of stands after repeated grazings.

Animal performance data on all new cultivars should be available prior to release. Animal evaluation is the last step in cultivar development prior to release.

Cultivar Release

The time taken to produce and release an improved endophyte-free cultivar can be 10 yr or longer. It is not difficult to produce a cultivar free from the endophyte since procedures are readily available to remove the endophyte from the seed. Considerable time is expended,

Table 3. Average daily gains of cattle grazing endophyte-free tall fescue cultivars and experimental synthetics.

Entry	Year			
	1979	1980	1981	1982
	lb/d			
H-1	0.94b*	1.48a	1.28a	1.72bc
WG3B	0.95ab	1.38a	1.38a	1.62c
WG2B	0.89b	1.33a	1.43a	1.87ab
Mozark	1.18a	1.50a	1.41a	1.88ab
Coefficient of variation (%)	11.5	24.9	12.1	6.1

* Entries in a column followed by the same letter are not significantly different from each other at $P = 0.05$ according to Duncan's multiple range test.

however, in completing cycles of selection for the incorporation of desirable agronomic traits and in the testing phases.

State and federal laws have been established to produce and maintain pure forage grass seed, including cultivars of tall fescue. The Federal Seed Act in combination with state seed laws, requires that a label be attached to or printed on each sack of seed sold. The label includes the name of the crop, percentage of pure seed, inert matter, other crop seed, weed seed, and germination. In addition, several states are requiring that either the percent of viable endophyte or total endophyte (not specifying if it is alive or dead) be included on the label. Seed lots found in violation may be withdrawn from sale and relabelled and legal action taken against the seed merchandiser.

FUTURE

Eliminating the endophyte from tall fescue cultivars has greatly improved forage quality and animal performance. The future looks encouraging for additional improvement of forage quality by selecting for quality attributes. Sleper (1985) reported on selecting tall fescue for improved in vitro dry matter disappearance (IVDMD) in the fall on plants that were 6 in. high. After three cycles of differential selection for IVDMD, the third cycle low-IVDMD population had a mean value of 79.8%, while the third cycle high-IVDMD population had a mean value of 84.0%. Slight progress has apparently been made in divergent selection for IVDMD.

Because the endophyte has apparently provided some protection from attack by such pests as insects and nematodes, the breeding effort may become involved with finding additional types of genetic pest resistance. This is important in persistence of tall fescue pastures.

It is well recognized that the endophyte confers important biological properties to the tall fescue plant. Another approach to tall fescue improvement may be to genetically modify the fungus to remove the hazards to grazing animals while maintaining the factor beneficial to the tall fescue. It is possible that the future may hold genetic alteration of the endophyte itself, as well as its host.

SUMMARY

Breeding endophyte-free tall fescue will undoubtedly continue to gain emphasis in the coming years. The techniques used for tall fescue improvement will remain essentially the same as in the past, with one major addition. The endophyte will have to be eliminated from the grass prior to testing and evaluation.

Removing the endophyte from tall fescue increases opportunities for breeders, and ultimately for forage producers. Potential new challenges, such as increasing stand persistence in marginal areas of adaptation, are even now being addressed. Perhaps more importantly, the challenge of further increasing the usefulness of the species through quality improvement, physiological alteration, etc., is also being met.

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