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Endophytes of perennial ryegrass and tall fescue

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Endophytes are fungi that live between the plant cells of many forage grasses. Their presence within the plant is symptomless, living mostly near the base of the grass tiller and in the growing points, spreading upwards as the seed heads develop in reproductive tillers. Smaller amounts of endophyte can be found in leaf blades and sometimes trace amounts can be found in roots. Their relationship with the host grass is symbiotic – the endophyte obtains its nutrition and spread (via seed) from the grass and the grass obtains a number of benefits from alkaloids produced. In a grazing situation, some of the alkaloids benefit the system, but others may result in a range of animal disorders.

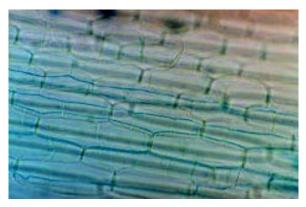
Most interest and research has been into *Neotyphodium* spp. endophytes in perennial ryegrass (*Lolium perenne*, endophyte *N. Iolii*) and tall fescue (*Festuca arundinaceae*, endophyte *N. coenophialum*). Endophytes infecting these grasses are either naturally occurring 'wild type' or a selected or novel endophyte – selected according to the range of alkaloids produced and then inoculated into the grass species. The endophytic fungi were initially identified in 1933 and subsequent research found that infected plants were more persistent and productive because of greater resistance to pests (insects and nematodes) and greater tolerance of drought.

Perennial ryegrass, tall fescue and turf tall fescues containing either wild or select endophytes have been more persistent and productive in areas of NSW where African Black Beetle (*Heteronychus*

arator), Pruinose and Dusky Pasture Scarabs Sericesthis sp.) are found on the tablelands and coastal areas. The most important areas where positive advantage from using cultivars containing endophyte have been identified to date include the South Coast from Nowra to the Victorian border and the Southern Highlands.

Disadvantages of cultivars with wild endophyte

Some alkaloids produced by the grass/endophyte association have been linked with animal production and health problems. The incidence of these problems is seasonal, and their severity highly variable. In some years no problems are encountered but in other years several problems may occur. Problems are most likely when livestock are grazing perennial ryegrass or tall fescue dominant pastures containing 'wild type' endophytes.



Endophyte (blue lines) growing between plant cells in perennial ryegrass. Source: W. Wheatley

The problem specific to endophyte infected perennial ryegrass is a nervous disorder called 'ryegrass staggers', which can occur in horses, cattle, sheep, alpacas and deer; it does not occur with tall fescue. The problem specific to tall fescue is a lameness disorder called 'fescue foot', which has been known to occur in cattle and sheep. It is theoretically possible that perennial ryegrass could also produce this disorder, but so far it has never



been substantiated. There are a group of problems that can potentially occur with either grass, these being referred to collectively as 'summer ill thrift'. The individual problems that contribute to this ill thrift syndrome are:

- reduced appetite or reduced feed intake;
- reduced live weight gain or weight loss;
- reduced milk flow or reduced milk production:
- an abnormal increase in body temperature (heat stress or hyperthermia); and
- mild intestinal inflammation resulting in diarrhoea (hence faecal dags) in a few animals.

The ill thrift problem has been most commonly encountered overseas with tall fescue. It is possible that it also occurs sometimes with perennial ryegrass, but because its occurrence will tend to coincide with outbreaks of 'ryegrass staggers' it may be overlooked or inadvertently regarded as part of that problem.

Some alkaloids produced by the grass/endophyte association have been linked with animal production and health problems. Most evidence relates to tall fescue rather than perennial ryegrass, and much of it is associated with problems in horses, rather than sheep or cattle. The specific effects on reproductive performance that might potentially occur, in some seasons, are:

- failure of embryos to implant;
- · death of early stage embryos;
- late term abortions;
- small birth weights;
- an increased number of stillbirths: and
- an increase in neonatal deaths within the first 24 hours of birth.

Alkaloids produced by endophytes

There are many substances produced in varying amounts by different endophytes. So far four substances have been identified that are of interest. The effects of these endophyte toxins on livestock, predatory insects and infectious diseases of ryegrass and fescue are not yet fully understood.

Lolitrem B is a cause of ryegrass staggers. It can be found concentrated at the base of the rvegrass plant in the leaf sheath and also the seed head in reproductive tillers. Concentration increases when the plant is stressed for moisture or when soil nitrogen is high. Significant concentrations of this substance will only develop during summer and autumn, and only in some years. Perennial ryegrass hay or silage harvested during these periods could potentially contain significant amounts of this toxin, but if fed out at a level that is less than 50% of the total dry matter on offer in the

daily ration they are unlikely to cause a clinical problem.

Ergovaline can cause fescue foot, summer ill thrift, or reproductive problems. Ergovaline concentrations increase when either tall fescue or perennial ryegrass are stressed for moisture or when soil nitrogen is high. Significant concentrations of this substance will only develop during summer and autumn, and only in some years. Hay or silage harvested during these periods can potentially contain significant amounts of this toxin, but if fed out at a level that is less than 50% of the total dry matter on offer in the daily ration they are unlikely to cause a clinical problem.

However, ergovaline protects tall fescue and perennial ryegrass from the damage caused by the African black beetle and the Argentinian stem weevil (a very minor pest of pastures in southeastern NSW. A very similar toxin to ergovaline can sometimes be produced on the seed heads of either tall fescue or perennial ryegrass by a fungus called Claviceps purpurea. This is the 'ergot' fungus and its toxin is called ergotamine. This toxin will cause identical effects in livestock to ergovaline.

Peramine can occur in both endophyte infected perennial ryegrass and tall fescue. It is not known to cause any deleterious effects in livestock. It can deter Argentinian stem weevil but it has no effect on the African black beetle. The full range of insects affected is yet to be determined.

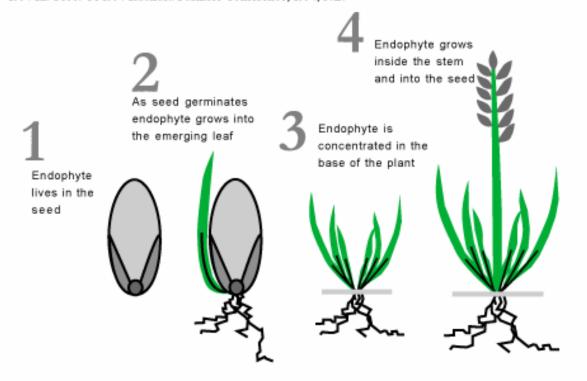
Loline (also known as Festucine) is a substance with insecticidal properties; it deters insects from feeding on plants. It is found in tall fescue and some species of annual ryegrass but has not yet been identified in perennial ryegrass.

Endophyte types

Wild endophytes refer to naturally occurring types that can be found in fescue and ryegrass. They produce variable concentrations of alkaloids.

Select or novel endophytes are naturally occurring endophytes that are selected and put into perennial ryegrass or tall fescue varieties because they produce lower or nil levels of one or more alkaloids and are non-toxic or have less toxicity to livestock. Some select endophytes do not provide the same insect protection as do wild types. The select endophyte lines presently available include AR1 developed by AgResearch, New Zealand. This is available in several perennial ryegrass cultivars. It relies on peramine to confer insect resistance and produces no ergovaline or lolitrem B. Tolosa, perennial ryegrass, contains NEA2 endophyte. This produces peramine and ergovaline, but has a reduced level of lolitrem B production, hence a lower risk of causing ryegrass staggers.

Figure 1. Life cycle of endophyte in perennial ryegrass Source: adapted from RA Prestidge and ER Thom Facts about endophytes 1994 In: Proc. Of 1994 Ruakura Farmers Conference, 1994, NZ.



Selected endophytes are presently used in tall fescues in the USA under the trade name Max Q and will be available from 2003 in some Australian tall fescue cultivars under trade name of Max P. These produce peramine and loline but no ergovaline.

Loline occurs in the roots of the tall fescue providing protection from root-feeding African black beetles and other yellow-headed pasture cockchafers. Cultivars with selected endophytes are proving more productive and considerably more persistent on the South Coast.

It should be noted that the endophyte/cultivar association is individualistic. When selected endophytes are introduced into a number of cultivars of, say perennial ryegrass, the level of toxin produced within each cultivar may vary considerably. Consequently careful testing is required to ensure that a particular cultivar is both safe for livestock and capable of reasonable pasture persistence.

Life history

Endophyte fungi live in the seed of infected plants. As seed germinates it grows into the emerging leaf. When the plant is vegetative, endophytes are found in the leaf sheath and so mainly near the base of the plant. In spring when reproductive tillers are produced it grows up inside the stein and into the seed. See Figure 1.

Endophyte in tall fescue

Australian and New Zealand tall fescues listed in Pasture Grass, Legume and Herb Varieties used in NSW 2002-2003 (NSW Agriculture (now NSW DPI) and Grasslands Society of NSW publication) contain no wild endophyte, however some cultivars used in the past in NSW pastures, all cultivars used for turf, and many cultivars from overseas, contain endophyte.

Endophyte in perennial ryegrasses

The majority of older cultivars of perennial ryegrass contain 'wild type' endophytes although some cultivars have low rates of seed infection and seed of many cultivars can now be purchased with low or nil endophyte infection levels.

Seed infection

Endophytes are only transferred from one generation to the next by seed infection. In New Zealand, seed lines infected with endophyte are considered to be high when 70% or more seed is infected with live endophyte. To be considered 'low', seed infection must be less than 25%. Most perennial ryegrasses can also be purchased with nil endophytes, which in New Zealand is 5% or less of the seeds with live endophytes.

These are New Zealand standards; currently no Australian standard for endophytes in seed exists.

Seed storage

Survival of endophytic fungi in seed declines as seed storage time increases. This decline occurs more rapidly as temperature and humidity in storage increases. Quite importantly, declines in live endophyte occur at a faster rate than declines in seed germination.

To maintain endophytes in seed during storage, temperatures should be 5°C or less and/or be low moisture seed (below 11%) and stored in moisture proof bags to maintain a low seed moisture content.

If buying high endophyte lines or the newer select or novel endophyte lines, in perennial ryegrass or tall fescue, it is important to check with the seller that the seed is fresh and that the endophyte level is still as stated.

Managing endophytes on farms

One option is not to grow perennial ryegrass or tall fescue that contains 'wild endophyte', that is, to use a nil endophyte line and accept a shorter potential pasture life. This affords maximum safety in terms of livestock production and health.

Another option is to grow perennial ryegrass with high 'wild endophyte' perennial ryegrass types to maximise pasture persistence, and then to manage them so as to reduce the risk of animal production and health issues. This is certainly valid in areas known to have regular problems with African black beetle on the South Coast of NSW, or on the tablelands of NSW where yellow headed cockchafers can restrict pasture life. In this situation the risk of negative animal effects can be minimised by:

- ensuring pastures contain other species such as other perennial grasses, clover or herbs;
- · providing alternative feed during summer and autumn months when alkaloid content is highest;
- slashing pastures to remove seed heads:
- · avoiding close grazing after the autumn break to prevent stock eating the leaf sheath;
- · avoiding nitrogen applications in summer and autumn when higher alkaloid concentrations are possible.

Tall fescues containing 'wild endophytes' should not be grown for animal production. The final option is to grow cultivars of perennial ryegrass or tall fescue that are infected with selected endophyte strains that have reasonable pasture persistence but a reduced risk of negative animal effects.

Sowing select or nil endophyte lines

If sowing select or nil endophyte cultivars into soil previously sown to wild endophyte types it is

important to reduce the residual seed bank in the soil and kill all the existing ryegrass plants to prevent contamination of the new pasture. Surveys of perennial ryegrass growing areas in NSW, Victoria and Tasmania have shown that older ryegrass pastures generally have a very high proportion of endophyte infected plants (because the nil endophyte plants have died out).

Sowing low, nil or select endophyte cultivars into soil with high 'wild endophyte' seed contamination will result in contamination of the new pasture and eventually the entire pasture will become a high endophyte dominant sward.

Residual seed is best removed by growing at least two or more forage crops prior to the pasture, or using a long chemical fallow to reduce seed reserves.

Testing forage for lolitrem B and ergovaline

Some laboratories offer (for a fee) a testing service for measuring lolitrem B and ergovaline concentrations in forage. These tests can be conducted on freshly growing perennial ryegrass or tall fescue pasture samples, or on samples of hay or silage made from these grasses. The results of these tests should be interpreted with caution.

The mere presence of these compounds in a feed sample does not necessarily imply that livestock eating them will experience any negative production or health effects. Currently, differences of opinion exist over what concentration of each substance needs to be present before negative production or health effects on livestock are likely to occur.

The most reliable scientific evidence would suggest that for sheep and cattle a plant ergovaline concentration of between 0.75 and 1.25 mg per kg dry matter can pose a risk, when that foodstuff is the only feed being eaten.

Likewise for lolitrem B, the risk concentration is between 1.5 and 2.5 mg per kg dry matter. If the affected foodstuff makes up less than 50% of the daily ration then the dilution effect may be considerable and the risk correspondingly negligible.

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Disclaimer: The information contained in this publication is based on knowledge and understanding at the time of writing (February 2007). However, because of advances in knowledge, users are reminded of the need to ensure that information upon which they rely is up to date and to check currency of the information with the appropriate officer of New South Wales Department of Primary Industries or the user's independent adviser.

PASTURE IMPROVEMENT CAUTIONS

Pasture improvement may be associated with an increase in the incidence of certain livestock health disorders. Livestock and production losses from some disorders are possible. Management may need to be modified to minimise risk. Consult your veterinarian or adviser when planning pasture improvement.

The Native Vegetation Act 2003 restricts some pasture improvement practices where existing pasture contains native species. Inquire through your office of the Department of Natural Resources for further details.

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