The role of *Epichloë* grass endophytes during pasture renewal

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Highlights

The literature on the importance of *Epichloë* grass endophytes during pasture renewal is reviewed. Perennial ryegrass endophyte strains such as AR1, NEA2, and Standard Endophyte (SE) as well as tall fescue and meadow fescue endophytes, significantly increase seedling survival at establishment under insect pressure, for example from adult Argentine stem weevil, grass grub, and African black beetle. However, in endophyte-infected ryegrass, insect-derived plant damage increases 10-43 days after sowing despite the presence of endophyte. Insecticidal seed treatments can mitigate the vulnerability to insect predation during this time.

Keywords: African black beetle, Argentine stem weevil, fungal alkaloids, grass grub, seedling establishment

Background

New Zealand's temperate climate enables pastoral-based livestock farming throughout the year. Pasture renewal is a major component of farm management practices that increase pasture and farm production. However, over time pastures can deteriorate due to a combination of factors such as weed infestation, treading damage by livestock, poor persistence of sown species, drought, flooding, or pests and diseases. Such pastures are typically removed using herbicides (e.g., glyphosate) and resown with cultivars of grasses, legumes and/or herbs to restore pasture productivity and deliver the benefits of improved genetics (Bryant et al. 2010). Different techniques can be used to renew pastures, including cultivation and cropping before sowing the seed, or from 'grass to grass', for which the seed is drilled or broadcast (Stewart et al. 2014b). The economic value of pasture renewal is estimated to be $900/ha annually through improved feed supply and animal production (Glassey et al. 2010).

Over 80% of ryegrass seed sold in New Zealand is infected with a fungal endophyte (*Epichloë festucae* var. *lolii*) (Milne 2007; Caradus & Johnson 2019). These fungi are maternally transmitted via seed and live between the plant cells of many cool-season grasses, including perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*), two important grass species in intensively managed, temperate pastures worldwide. The host grass and the endophyte live in a mutualistic relationship, in which both partners benefit. The grass supplies the endophyte with a habitat, nutrients, and a chance to propagate. In turn, the endophyte enhances the plant's competitive ability to cope with biotic (Popay 2009) and abiotic (Malinowski & Belesky 2000) stresses through the production of secondary metabolites (alkaloids). Some of these alkaloids strongly deter herbivorous insect pests (Prestidge & Van der Zijpp 1988), while other alkaloids can also have negative impacts on grazing livestock (Fletcher & Harvey 1981). The perennial ryegrass endophyte, which became naturalised in New Zealand, referred to here as New Zealand Standard Endophyte (SE), produces three main types of alkaloids: lolitrems, ergot alkaloids, and peramine (Johnson et al. 2013). Loline alkaloids are produced by the tall fescue endophyte (*E. coenophiala*), as well as peramine, and the meadow fescue endophyte (*E. uncinata*). Alkaloids are expressed in various combinations and concentrations, which are dependent on endophyte strain and influenced by grass genotype and environmental conditions (Spiering et al. 2005). The alkaloid compound and *in planta* concentration and distribution are the main drivers determining the toxicity to grazing livestock and/or insect pests. For example, ergovaline and peramine are highly active feeding deterrents to insects such as, respectively, African black beetle (*Heteronychus arator*) and Argentine stem weevil (ASW, *Listronotus bonariensis*), two major pests in improved New Zealand pastures (Rowan & Latch 1994; Ball et al. 1997a). Antibiosis caused by the loline-producing endophytes affects a range of insects, including grass grub (*Costelytra giveni;* previously called *C. zealandica*) (Patchett et al. 2011) and ASW larvae (Jensen et al. 2009). The identification of the

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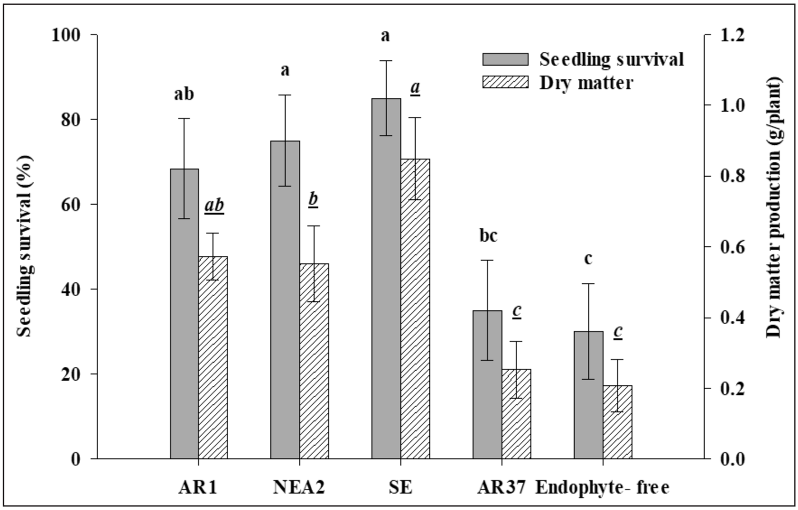

Figure 1 Seedling survival (%) and mean dry matter (DM) production (g/plant±SEM) of 64-day old perennial ryegrass plants infected with endophyte strains AR1, NEA2, Standard endophyte (SE), AR37, or endophyte-free after exposure to adult Argentine stem weevil when seedlings were 7-22 days old. Dead plants were excluded from DM production analysis. NEA2 contains a mix of two endophyte strains NEA2 and NEA6. For each variable, mean values with the same letter are not significantly different at P<0.05. Graph redrawn from Ruppert et al. (2017).

role of alkaloids has led to exploration of the use of *Epichloë* endophytes in integrated pest management in pastures. Selected endophyte strains, which retain insect resistance, but do not cause mammalian toxicity (or have only low levels of problematic alkaloids), have been available commercially since the early 2000s via seed, contributing an estimated $200M annually to New Zealand's pastoral economy (Johnson et al. 2013). These commercial endophyte strains include AR1, AR37, NEA strain mixtures and Edge for perennial ryegrass, MaxP® (AR542/AR584) for tall fescue, GrubOut U2 for *Festulolium* (hybrid of *Festuca* and *Lolium*), and MaxR™ for meadow fescue (*Festuca pratensis*). Here we review the literature on the role of *Epichloë* grass endophytes during pasture renewal.

Importance of pasture establishment

Pastures are most vulnerable during the early establishment phase when insect predation can have significant long-term effects on plant community composition, productivity and pasture resilience. Their small size and palatability make seedlings an attractive food source for many herbivores. For example, adult ASW can seriously damage developing perennial ryegrass seedlings during pasture renewal. Adult weevils feed on the emerging shoot towards the base of the plant often completely severing the emerging shoot near the soil surface, causing plant death or severe stunting (Pottinger 1961; Ruppert et al. 2017). Furthermore, African black beetle, grass grub, and grey field slugs (*Deroceras reticulatum*) can severely damage young grass seedlings, reducing plant vigour or even causing establishment failure (Popay & Baltus 2001; Popay & Tapper 2007; Wilson & Barker 2011). Farmers expect the sown species to be dominant, contributing significantly to dry matter (DM) production. However, poor pasture establishment can lead to gaps in the sward that can be occupied by weeds which can eventually become dominant (Tozer et al. 2011). Successful seedling establishment during pasture renewal is therefore a crucial initial component of productive, persistent, and resilient pastures.

Role and efficacy of endophyte strains in seedlings

Endophyte alkaloid concentrations in seeds can be up to 13-fold greater than concentrations in other parts of the grass (Ball et al. 1997b). Alkaloids in seeds are translocated into the emerging shoot upon germination, resulting in an initial concentration peak approximately 10 days after sowing (Hewitt et al. 2020). Insect pests such as adult ASW are able to distinguish between endophyte-infected and endophyte-free ryegrass seedlings and concentrate their feeding on those lacking the fungus (Stewart 1985). Argentine stem weevil seedling resistance is dependent on endophyte strains that express the fungal alkaloid peramine such as AR1, NEA endophyte strain seed mixes, and SE. Ryegrass seedlings infected with AR1 (cv. One50), NEA2 (cv. Trojan with a 50:50 mixture of endophyte strain NEA2 and NEA6) and SE were significantly less damaged by adult ASW, especially during the first week after sowing, resulting in increased survival and higher DM than seedlings without endophyte or seedlings infected with the non-peramine producing AR37 strain (Figure 1, Ruppert et al. 2017). However, measured in a separate experiment despite the presence of peramine, plant damage by adult ASW increased as the seedling matured from about 10-43 days after sowing (Figure 2, Ruppert et al. 2017).

The underlying reason for such seedling vulnerability to insect predation during pasture establishment is the dilution of alkaloids due to rapid seedling growth (Figure 2). Even though the endophyte starts its own alkaloid synthesis as early as 6 days after sowing (Hewitt et al. 2020), the developing seedling grows faster than new alkaloids are being produced or translocated. However, the vulnerability to adult ASW feeding lessens as the seedling matures and the level of damage becomes relatively minor in older seedlings. After the initial vulnerable phase, ryegrass seedling growth and alkaloid production are synchronised, and able to protect the mature plant against adult ASW predation (Hewitt et al. 2020).

Endophyte infection in tall fescue and meadow fescue also significantly increases seedling survival under grass grub attack (Figure 3), which is linked to the presence of loline alkaloids (Popay & Tapper 2007). It is unknown, but likely, that loline concentrations are diluted similarly, with seedling growth potentially leaving the seedling vulnerable until alkaloid production is synchronised with plant growth, as seen with ryegrass alkaloids.

Management strategies

Reducing insect damage to emerging seedlings is of high priority during pasture renewal. The management strategies depend on the history of the present pasture as well as understanding the insect behaviour. For instance, if the previous pasture was heavily damaged by African black beetle larvae and grass grub, the old insect infestation can carry over, resulting in high insect pressure on the emerging seedlings, especially when renewing from grass to grass. In the northern North Island, the normal time for pasture renewal in autumn coincides with the emergence of adult African black beetle, which can destroy young seed-

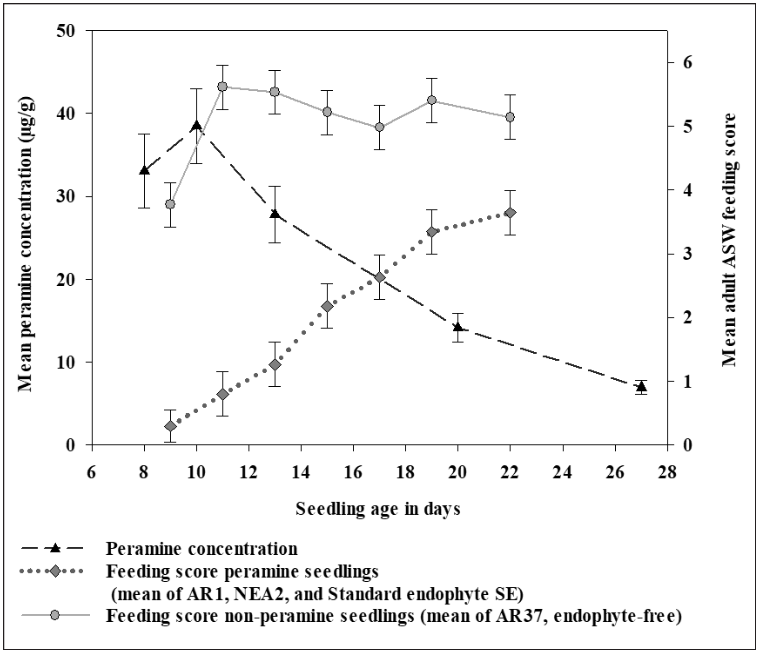

Figure 2 The relationship between peramine concentration and adult Argentine stem weevil (ASW) feeding in endophyte-infected perennial ryegrass seedlings during the early establishment phase. NEA2 contains a mix of two endophyte strains NEA2 and NEA6. Adult ASW feeding activity was scored on a scale of 0-6 whereby: 0 = no damage; 1 = little feeding, test nibbles can be seen; 3 = feeding on base of tiller; 4 = feeding on base and tip of tiller; 5 = seedling severed by chewing and detached from seed; 6 = seedling dead after extensive feeding. Vertical bars represent ± standard error of the mean. Graph redrawn from Ruppert (2016).

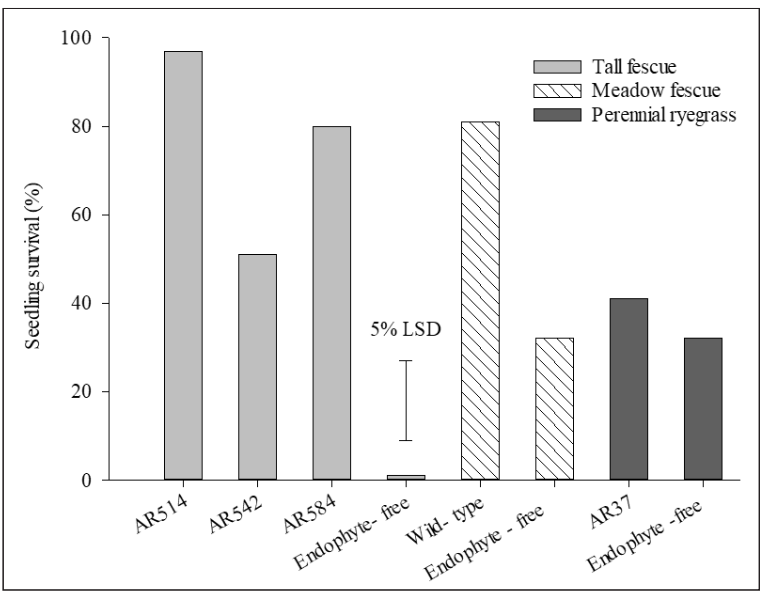

Figure 3 Seedling survival (%) of 15-day-old tall fescue, meadow fescue, and perennial ryegrass seedlings infected with different endophyte strains after grass grub feeding. Endophyte strains AR542 and AR584 are sold as MaxP® endophytes. Graph modified with permission from Popay & Tapper (2007).

lings (Popay & Baltus 2001; Bell et al. 2011). While high populations of African black beetle and grass grub can take several years to develop, adult ASW can invade new pastures quickly, especially when present in high numbers in the previous pasture. Several farm management strategies can be implemented, however, to reduce the risk of invertebrate damage and increase seedling establishment.

One of the most important management strategies alleviating insect damage during seedling establishment is the use of seeds coated with a systemic insecticide (such as Gaucho® a.i. imidacloprid or Poncho® a.i. clothianidin, Nichol & Walker 2013). Systemic insecticidal seed treatments or granules drilled along with the seed protect the emerging seedling from insect predation. Such treatments form a protective barrier around the seed, get taken up by the developing roots, and transported into the developing shoot. Even though insecticidal seed treatments are recommended for high seedling establishment (Nichol & Walker 2013), the active ingredients commonly used (e.g., in Poncho® or Gaucho®) belong to the neonicotinoids, a group of compounds associated with bee deaths and ecotoxic effects (Rundlöf et al. 2015). The use of neonicotinoids as seed treatments in integrated pest management has been debated intensely, especially in flowering plant species such as brassica and maize (Tooker et al. 2017). In general, grasses are wind and/or self-pollinated (Humphreys et al. 2010), and not a food source to bees. Therefore, neonicotinoid seed treatments of grass seeds are believed to be a minor threat to pollinators. However, only 1-10% of neonicotinoids applied to the seed get absorbed, while the rest remains in the soil where it poses a risk to beneficial insects and organisms as well as a risk of leaching into the groundwater (Krupke et al. 2017). For such reasons, neonicotinoids have been banned in the European Union since 2018 (European Commission 2018). Although appropriate seed treatments can improve the seedlings' ability to withstand insect attack, fungal endophytes remain a critical tool in improving pasture establishment, even though alkaloids are diluted by rapid seedling growth for a period of time after sowing leaving the seedling vulnerable to insect predation (Hewitt et al. 2020). The greatest risk of severe insect damage is when seedlings have emerged from the soil and are very young. At this stage the endophyte provides some protection, as shown here by the significantly higher survival rates of insecticide-free seedlings infected with peramine-producing endophyte strains (AR1: 68%, NEA2/NEA6: 75%, SE: 85%) compared with seedlings without endophyte (30%) or infected with a non-peramine producing endophyte strain (AR37: 35%) (Ruppert et al. 2017). Once seedlings are established after approximately 20 days, peramine-producing endophyte-infected plants are naturally able to withstand adult ASW attack as rapid plant growth compensates for their feeding. Although endophyte strain AR37 does not protect against adult ASW (Popay & Wyatt 1995), ryegrass plants with this endophyte are highly resistant to the larval stage (Popay et al. 2017) and to adult African black beetle but not larvae (Hume et al. 2007). Nevertheless, fungal endophytes do not match the effectiveness of insecticidal seed treatments. The consequences of such insecticidal treatments being withdrawn in New Zealand are understudied, increasing the pressure for pesticide manufacturers to find alternatives. Such alternative seed treatments may include synthetic insecticides such as chlorantraniliprole, which reduced armyworm feeding (*Mythimna unipuncta*) in corn seedlings (Carscallen et al. 2018).

Endophyte infection reduces slug populations in established perennial ryegrass and tall fescue swards (Pennell et al. 2018). However, it is unknown if endophyte infection protects the emerging seedling against slug feeding. The additional application of molluscicide during autumn sowing can reduce slug damage to emerging seedlings, especially after a wet summer (McCallum & Thomson 1990).

The timing of pasture renewal can also influence successful seedling establishment. Pastures are typically renewed in autumn and spring when temperatures and rainfall/irrigation facilitate rapid germination and plant growth. Insect damage during germination, such as with adult ASW, can be reduced when pastures are sown in autumn once egg-laying has finished and adults have entered reproductive diapause in which weevils are less active (Goldson & Penman 1979). This diapause is induced by photoperiod and is usually between early March and late July (Goldson 1981).

Soil cultivation before sowing may decrease the risk of insect damage to pastures. A study using different types of soil cultivation techniques showed that soil disturbance reduced grass grub numbers (Stewart 1986). Depending on soil type, deep soil cultivation such as ploughing is typically used following a crop, while direct drilling can be used in grass to grass renewal. There are various drawbacks to full cultivation, including comparatively high cost, longer waiting time before pasture can be grazed, and reduced environmental sustainability (less conserved soil moisture, deterioration of soil physical properties, organic carbon loss; e.g., Eijsackers 2011). In addition, although soil cultivation reduces the grass grub population to very low levels, it also reduces the natural pathogens which can regulate grass grub populations (Popay 1992). This often leads to an outbreak of grass grub 2-4 years later. Livestock treading and heavy rolling of the pasture in winter can reduce grass grub populations, which is attributed to soil movement/ disturbance and compaction. It has been suggested that reduced soil oxygen levels in these conditions may play a part in grass grub population reduction (Atkinson & Slay 1994).

Furthermore, if populations of pest insects have resulted in the need for pasture renewal, a break crop can reduce or eliminate them. Crop rotation removes the insect food source, naturally interrupting the insects' breeding cycle (Eerens et al. 2005). For example, if present pasture has been damaged severely by ASW, turnips can be used as a break crop to reduce insect pests and weed infestation (Gerard et al. 2009; Densley et al. 2011). In such instances it is not recommended to use Italian ryegrass (*Lolium multiflorum*) as a short-term break crop to feed stock during winter, as this ryegrass is a preferred host for ASW (Prestidge 1991). Argentine stem weevil will overwinter on this break crop and adult weevils invade new seedlings the following spring.

Conclusions

Seedling survival during pasture renewal has a major impact on pasture persistence and production. Newly sown pastures are especially vulnerable to insect predation, causing seedling death, which then can lead to open, weedy swards. Endophyte infection is an essential tool in minimising seedling damage of ryegrasses, tall fescue and meadow fescue in developing pastures. Peramine-producing ryegrass endophyte strains such as AR1, NEA2 and SE, and loline-producing tall fescue/ meadow fescue strains such as MaxP, can provide significant benefit by increasing pest resistance during seedling establishment via the presence of deterrent alkaloids. However, ryegrass seedlings with peramine-producing endophytes do become susceptible to insect feeding from 10-43 days after sowing due to a dilution-reduction in the peramine concentration and chemical defence as the seedling grows. After the initial vulnerable phase, ryegrass seedling growth and alkaloid production are synchronised, and able to protect the mature plant against insect predation depending on the endophyte strain and insect pest (Stewart et al. 2014a; Gange et al. 2019). It may be expected that loline alkaloids are similarly diluted with seedling growth potentially leaving the seedling vulnerable until alkaloid production is synchronised with plant growth as seen with ryegrass alkaloids, although this needs verification. Farmers are encouraged to be aware of local pests that affect seedling establishment and select suitable endophyte strains for their location. Additionally, insecticide-treated seeds should be used to ensure seedling survival, improving pasture resilience during the important establishment phase.

Further research is needed to i) develop effective seed treatments which do not contain ecotoxic compounds; ii) determine the potential of using summer crops as a sustainable management tool with the aim of reducing pest problems before autumn pasture renewal occurs; and iii) identify endophytes in a wider range of pasture grasses which are able to perform in a more variable and drier climate.

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REFERENCES

Atkinson DS, Slay MW. 1994. Winter management of grass grub (*Costelytra zealandia* (White)). *New Zealand Journal of Agricultural Research 37*: 553-558. doi:https://doi.org/10.1080/00288233.1994.9513094

Ball OJ-P, Miles CO, Prestidge RA. 1997a. Ergopeptine alkaloids and *Neotyphodium lolii*-mediated resistance in perennial ryegrass against adult *Heteronychus arator* (Coleoptera: Scarabaeidae). *Journal of Economic Entomology 90*: 1382-1391. doi:https://doi.org/10.1093/jee/90.5.1382

Ball OJ-P, Barker GM, Prestidge RA, Sprosen JM. 1997b. Distribution and accumulation of the mycotoxin lolitrem B in *Neotyphodium lolii*-infected perennial ryegrass. *Journal of Chemical Ecology 23*: 1435-1449. doi:https://doi.org/10.1023/B:JOEC.0000006474.44100.17

Bell NL, Townsend RJ, Popay AJ, Mercer CF, Jackson TA. 2011. Black beetle: lessons from the past and options for the future. In: Mercer CF. Ed. *Pasture Persistence Symposium. Grassland Research and Practice Series 15*. Dunedin, New Zealand: New Zealand Grassland Association, pp. 119-124. https://doi.org/10.33584/rps.15.2011.3193

Bryant JR, Lambert MG, Brazendale R, Holmes CW, Fraser TJ. 2010. Effects of integrated cropping and pasture renewal on the performance and profit of dairy farms. *Proceedings of the New Zealand Grassland Association 72*: 29-34. https://doi.org/10.33584/jnzg.2010.72.2813

Caradus JR, Johnson LJ. 2019. Improved adaptation of temperate grasses through mutualism with fungal endophytes. In: Schouten A. Ed. *Endophyte biotechnology: potential for agriculture and pharmacology*, Wallingford, United Kingdom: CABI, pp. 85-108. doi:https://doi.org/10.1079/9781786399427.0085

Carscallen GE, Kher SV, Evenden ML. 2018. Efficacy of chlorantraniliprole seed treatments against armyworm (*Mythimna unipuncta* [Lepidoptera: Noctuidae]) larvae on corn (*Zea mays*). *Journal of Economic Entomology 112*: 188-195. doi:https://doi.org/10.1093/jee/toy338

Densley RJ, Williams ID, Kleinmans JJ, McCarter SB, Tsimba R. 2011. Use of maize silage to improve pasture persistence in dairy farm systems: a review. In: Mercer CF. Ed. *Pasture Persistence Symposium. Grassland Research and Practice Series 15*. Dunedin, New Zealand: New Zealand Grassland Association, pp. 217-220. https://doi.org/10.33584/rps.15.2011.3205

Eerens JPJ, Hardwick S, Gerard PJ, Willoughby BE. 2005. Clover root weevil (*Sitona lepidus*) in New Zealand: the story so far. *Proceedings of the New Zealand Grassland Association 67*: 19-22. https://doi.org/10.33584/jnzg.2005.67.2588

Eijsackers H. 2011. Earthworms as colonizers of natural and cultivated soil environments. *Applied Soil Ecology 50*: 1-13. doi:https://doi.org/10.1016/j.apsoil.2011.07.008

European Commission. 2018. *Neonicotinoids*. Retrieved 30 October 2020 from: https://ec.europa.eu/food/plant/pesticides/approval_active_substances/approval_renewal/neonicotinoids_en.

Fletcher LR, Harvey IC. 1981. An association of a *Lolium* endophyte with ryegrass staggers. *New Zealand Veterinary Journal 29*: 185-186. doi:https://doi.org/10.1080/00480169.1981.34839

Gange AC, Currie AF, Spong JR. 2019. Endophytes as novel pest control Agents: myth or reality? In: Gange AC, Currie AF & Spong JR. Eds. *Endophyte Biotechnology: Potential for Agriculture and Pharmacology*. Wallingford, Oxfordshire, United Kingdom: CABI, pp. 68-84. doi:https://doi.org/10.1079/9781786399427.0068

Gerard PJ, Cooper BM, Eden TM, Howlett SA, Lane PMS, Panckhurst KA, Willoughby BE, Wilson DJ. 2009. Impact of ryegrass selection and paddock history on clover establishment in new dairy pasture. *Proceedings of the New Zealand Grassland Association 71*: 133-137. https://doi.org/10.33584/jnzg.2009.71.2759

Glassey CB, Roach CG, Strahan MR, McLean N. 2010. Dry matter yield, pasture quality and profit on two Waikato dairy farms after pasture renewal. *Proceedings of the New Zealand Grassland Association 72*: 91-96.

Goldson SL, Penman DR. 1979. Effect of time of sowing on Argentine stem weevil (*Hyperodes bonariensis* Kuschel) damage in autumn-sown Tama ryegrass. *New Zealand Journal of Agricultural Research 22*: 367-371. doi:https://doi.org/10.1080/00288233.1979.10430761

Goldson SL. 1981. Reproductive diapause in the Argentine stem weevil, *Listronotus bonariensis*, (Kuschel)(Coleoptera: Curculionidae), in New Zealand. *Bulletin of Entomological Research 71*: 275-287. doi:https://doi.org/10.1017/S0007485300008300

Hewitt KG, Mace WJ, McKenzie CM, Matthew C, Popay AJ. 2020. Fungal alkaloid occurrence in endophyte-infected perennial ryegrass during seedling establishment. *Journal of Chemical Ecology 46*: 410-421. doi:https://doi.org/10.1007/s10886-020-01171-9

Hume DE, Ryan DL, Cooper BM, Popay AJ. 2007. Agronomic performance of AR37-infected ryegrass in northern New Zealand. *Proceedings of the New Zealand Grassland Association 69*: 201-205. https://doi.org/10.33584/jnzg.2007.69.2673

Humphreys MO, Feuerstein U, Vandewalle M, Baert J. 2010. Ryegrasses. In: Boller B, Posselt UK & Veronesi F. Eds. *Fodder crops and amenity grasses*. New York, United States of America: Springer, pp. 211-260. doi:https://doi.org/10.1007/978-1-4419-0760-8_10

Jensen JG, Popay AJ, Tapper BA. 2009. Argentine stem weevil adults are affected by meadow fescue endophyte and its loline alkaloids. *New Zealand Plant Protection 62*: 12-18. doi:https://doi.org/10.30843/nzpp.2009.62.4800

Johnson LJ, De Bonth ACM, Briggs LR, Caradus JR, Finch SC, Fleetwood DJ, Fletcher LR, Hume DE, Johnson RD, Popay AJ, Tapper BA, Simpson WR, Voisey CR, Card SD. 2013. The exploitation of *epichloae* endophytes for agricultural benefit. *Fungal Diversity 60*: 171-188. doi:http://doi.org/10.1007/s13225-013-0239-4

Krupke CH, Holland JD, Long EY, Eitzer BD. 2017. Planting of neonicotinoid-treated maize poses risks for honey bees and other non-target organisms over a wide area without consistent crop yield benefit. *Journal of Applied Ecology 54*: 1449-1458. doi:https://doi.org/10.1111/1365-2664.12924

Malinowski DP, Belesky DP. 2000. Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. *Crop Science 40*: 923-940. doi:https://doi.org/10.2135/cropsci2000.404923x

McCallum DA, Thomson NA. 1990. Effect of a molluscicide and an insecticide on the establishment of direct-drilled ryegrass, tall fescue and phalaris. *Proceedings of the New Zealand Grassland Association 52*: 237-240. https://doi.org/10.33584/jnzg.1990.52.1940

Milne GD. 2007. Technology transfer of novel ryegrass endophytes in New Zealand. In: Popay AJ & Thom ER. Eds. *6th International Symposium on Fungal Endophytes of Grasses. Grassland Research and Practice Series 13*. Dunedin, New Zealand: New Zealand Grassland Association, pp. 237-239.

Nichol WW, Walker SF. 2013. The effect of novel endophyte and insecticide seed treatment on the establishment of long rotation or perennial ryegrass plants in the presence of adult Argentine Stem Weevil (*Listronotus bonariensis*). In: Michalk DL, Millar GD, Badgery WB & Broadfoot KM. Eds. *Proceedings of the 22nd International Grasslands Congress (Revitalising Grasslands to Sustain Our Communities)*. Orange, New South Wales, Australia: New South Wales Department of Primary Industries, pp. 1627-1628.

Patchett BJ, Gooneratne RB, Chapman B, Fletcher LR. 2011. Effects of loline-producing endophyte-infected meadow fescue ecotypes on New Zealand grass grub (*Costelytra zealandica*). *New Zealand Journal of Agricultural Research 54*: 303-313. doi:https://doi.org/10.1080/00288233.2011.608686

Pennell CG, Rolston MP, van Koten C, Mace WJ, Hume DE, Card SD. 2018. The effect of grass endophytes on earthworms and slugs under a turf mowing regime. *New Zealand Plant Protection 71*: 240-247. doi:https://doi.org/10.30843/nzpp.2018.71.148

Popay AJ. 1992. Population regulation of *Costelytra zealandica* by pathogens in the North Island of New Zealand. In: Jackson A & Glare TR. Eds. *Use of pathogens in scarab pest management*. Andover, Hampshire, United Kingdom: Intercept Ltd, pp. 141-152. doi:https://doi.org/10.1017/S0007485300041882

Popay AJ, Wyatt RT. 1995. Resistance to Argentine stem weevil in perennial ryegrass infected with endophytes producing different alkaloids. *Proceedings of the New Zealand Plant Protection Conference 48*: 229-236. https://doi.org/10.30843/nzpp.1995.48.11487

Popay AJ, Baltus JG. 2001. Black beetle damage to perennial ryegrass infected with AR1 endophyte. *Proceedings of the New Zealand Grassland Association 63*: 267-271. https://doi.org/10.33584/jnzg.2001.63.2415

Popay AJ, Tapper BA. 2007. Endophyte effects on consumption of seed and germinated seedlings of ryegrass and fescue by grass grub (*Costelytra zealandica*) larvae. In: Popay AJ & Thom ER. Eds. *6th International Symposium on Fungal Endophytes of Grasses. Grassland Research and Practice Series 13*. Dunedin, New Zealand: New Zealand Grassland Association, pp. 353-356.

Popay AJ. 2009. Insect herbivory and defensive mutualisms between plants and fungi. In: White JF, Jr. & Torres MS. Eds. *Defensive Mutualism in Microbial Symbiosis*. Boca Raton, Florida, United States of America: CRC Press, pp. 347-366. doi:https://doi.org/10.1201/9781420069327

Popay AJ, Rijswijk K, Goldson SL. 2017. Argentine stem weevil: farmer awareness and the effectiveness of different ryegrass/endophyte associations. *Journal of New Zealand Grasslands 74*: 23-28. doi:https://doi.org/10.33584/jnzg.2017.79.570

Pottinger R. 1961. *A study on the biology and economic importance of the Argentine stem weevil, Hyperodes bonariensis kuschel, in New Zealand*. MAgSc thesis, Canterbury Agricultural College, University of New Zealand.

Prestidge RA, Van der Zijpp S. 1988. The distribution of endophytes in pasture grasses and the interaction with Argentine stem weevil. In: Stahle PP. Ed. *Proceedings of the 5th Australasian Conference on Grassland Invertebrate Ecology*. Melbourne, Australia. Pp. 20-27.

Prestidge RA. 1991. Susceptibility of Italian ryegrasses (*Lolium multiflorum* Lam.) to Argentine stem weevil (*Listronotus bonariensis* (Kuschel)) feeding and oviposition. *New Zealand Journal of Agricultural Research 34*: 119-125. doi:https://doi.org/10.1080/00288233.1991.10417801

Rowan DD, Latch GCM. 1994. Utilization of endophyte-infected perennial ryegrasses for increased insect resistance. In: Bacon CW & White JF, Jr. Eds. *Biotechnology of Endophytic Fungi of Grasses*. Boca Raton, Florida, United States of America: CRC Press, pp. 169-183. doi:https://doi.org/10.1201/9781351070324

Rundlöf M, Andersson GKS, Bommarco R, Fries I, Hederström V, Herbertsson L, Jonsson O, Klatt BK, Pedersen TR, Yourstone J. 2015. Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature 521*: 77-80. doi:https://doi.org/10.1038/nature14420

Ruppert KG. 2016. *Fungal alkaloid occurrence during seedling establishment and early growth in Lolium perenne seedlings infected with Epichloë festucae var. lolii and the influence of adult Argentine stem weevil (Listronotus bonariensis) feeding on alkaloid concentrations*. MSc thesis, Massey University, Palmerston North, New Zealand. 126 p.

Ruppert KG, Matthew C, McKenzie CM, Popay AJ. 2017. Impact of *Epichloë* endophytes on adult Argentine stem weevil damage to perennial ryegrass seedlings. *Entomologia Experimentalis et Applicata 163*: 328-337. doi:https://doi.org/10.1111/eea.12584

Spiering MJ, Lane GA, Christensen MJ, Schmid J. 2005. Distribution of the fungal endophyte *Neotyphodium lolii* is not a major determinant of the distribution of fungal alkaloids in *Lolium perenne* plants. *Phytochemistry 66*: 195-202. doi:https://doi.org/doi:10.1016/j.phytochem.2004.11.021

Stewart AV. 1985. Perennial ryegrass seedling resistance to Argentine stem weevil. *New Zealand Journal of Agricultural Research 28*: 403-407. doi:https://doi.org/10.1080/00288233.1985.10430445

Stewart AV, Kerr GA, Lissaman W, Rowarth JS. 2014a. Chapter 8. Endophyte in ryegrass and tall fescue. In: Davis K & Casey M. Eds. *Pasture and forage plants* *for New Zealand. Grassland Research and Practice Series 8 4th ed*. Dunedin, New Zealand: New Zealand Grassland Association, pp. 66-77.

Stewart AV, Kerr GA, Lissaman W, Rowarth JS. 2014b. Chapter 3. Pasture renewal techniques. In: Davis K & Casey M. Eds. *Pasture and forage plants for New Zealand. Grassland Research and Practice Series 8 4th ed*. Dunedin, New Zealand: New Zealand Grassland Association, pp. 17-21.

Stewart KM. 1986. Control of grass grub (*Costelytra zealandica*) by cultivation in spring or summer. *New Zealand Journal of Experimental Agriculture 14*: 83-87. doi:https://doi.org/10.1080/03015521.1986.10426129

Tooker JF, Douglas MR, Krupke CH. 2017. Neonicotinoid seed treatments: limitations and compatibility with integrated pest management. *Agricultural & Environmental Letters 2*: 1-5. doi:https://doi.org/10.2134/ael2017.08.0026

Tozer KN, Cameron CA, Thom ER. 2011. Pasture persistence: farmer observations and field measurements. In: Mercer CF. Ed. *Pasture Persistence Symposium. Grassland Research and Practice Series 15*. Dunedin, New Zealand: New Zealand Grassland Association, pp. 25-30. https://doi.org/10.33584/rps.15.2011.3216

Wilson MJ, Barker GM. 2011. Slugs as pasture pests. In: Mercer CF. Ed. *Pasture Persistence Symposium. Grassland Research and Practice Series 15*. Dunedin, New Zealand: New Zealand Grassland Association, pp. 241-246. https://doi.org/10.33584/rps.15.2011.3194