The effects of clover and nitrogen fertiliser on the presence of pasture pests in dairy pastures

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Highlights

A comprehensive dataset obtained from sampling four trials investigating interactions between perennial ryegrass and white clover under two levels of applied nitrogen (N) has highlighted different effects of pasture composition and N use on pasture pests.

- For swards with white clover, presence of clover root weevil, whitefringed weevil and grass grub increased under low N by 36%, 11% and 5%, respectively, compared with high N treatments.
- High N increased the presence of both Argentine stem weevil and root aphid by 7%.
- Unexpectedly, clover reduced the presence of two grass feeders, Argentine stem weevil and black beetle, by 7% and 11% respectively.
- Presence of grass grub was 17-30% lower under tetraploid ryegrasses than under diploids.
- More clover and reduced N fertiliser inputs could reduce the frequency of black beetle and Argentine stem weevil with environmental benefits.

Keywords: environmental constraints, host plants, insect nutrition, macronutrients, pest management

Background

As individual species and collectively, pasture pests have a major impact on pasture persistence and are often difficult to control. Reducing pest populations will build resilience in perennial ryegrass (*Lolium perenne*) - white clover (*Trifolium repens*) pastures that will improve their productivity and persistence, but this requires an understanding of the main factors that influence these insects such as pasture composition and nutrients. At the same time, a balance is required between these factors and the pasture growth needed to deliver the best outcome for livestock production and the environment. Here we summarise the overall effects of clover and nitrogen (N) fertiliser on the presence of different insect pests in a series of trials that investigated grass-clover interactions, under two levels of N at four sites (Waikato, Manawatu, Canterbury and Southland) across New Zealand. The main purpose of these trials was to determine if clover affected dry matter yields to the extent that adjustments to the Forage Value Index would be required. The trials compared yields of eight perennial ryegrass cultivars, with and without clover and at two levels of applied N. Full details of the trial methodology and results for each site, together with a cross-site analysis and effects on metabolisable energy of the different treatments, have been reported (Chapman et al. 2018a, b, c; Cosgrove et al. 2018a, b; Lee et al. 2018; Stevens et al. 2018). Annual sampling of each trial by soil coring was conducted in early autumn of each of the 4 years for enumeration of the invertebrates present. This was important for understanding the role pests had on the dynamics of the ryegrass-clover interactions and impact of N after pasture renewal. The trials have provided the most comprehensive data set ever gathered on New Zealand pasture pests.

Insect herbivory and pasture pests

Insect herbivores are highly dependent on the nutrients provided by their host plant for growth and development. In general, their body tissues contain greater amounts of N than the plants they feed on and this may limit their growth or require increased consumption to compensate for the difference (Huberty & Denno 2006; Deans et al. 2015). Although much of the research on insect nutrition has focused on the importance of N (e.g., Mattson 1980), more recent research has shown that carbohydrate/protein ratios are more important for growth and development. Additional nutrients, including N, do not necessarily equate with increased growth and reproduction of insects, and indeed may be harmful to them if this does not meet their macronutrient requirements (Joern et al. 2012). Within pastures there is also temporal and spatial heterogeneity of plant species, particularly clover, which means that mobile insects on the surface can self-select a diet that optimises their nutrition whereas sub-surface feeders are more constrained by a

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limited ability to move. Thus, the effects of N fertiliser and clover presence potentially have major and varied consequences for different pasture pests.

Pasture pests are estimated to cost New Zealand farmers between $1.7B and $2.3B annually through reduced pasture production (Ferguson et al. 2018). This estimate does not include costs of renewal of pastures which are often runout due to the effects of pests, weed ingress and disease. Management of some of our most destructive pests such as grass grub (*Costelytra giveni*), remains a major challenge for farmers. Solutions for other serious pests include the use of endophyte and introduced biological control agents.

One of the first steps to successful pest management is to know which insects are prevalent in different parts of the country, thereby enabling farmers to make optimum decisions regarding their management. Using the data obtained from four consecutive annual samplings of the trials, the presence of each pest species was calculated as the percentage of core samples taken that contained that species for each calendar year and according to the age of the trial. This accounted for a timing difference for the Southland site, which was planted in December 2012, in contrast to the other sites which had been established the previous autumn. As for the other sites, the Southland site was sampled in autumn 2013 but in contrast to the other sites an additional sampling was also undertaken in 2017 which was then regarded as the Year 4 sampling. Thus, data are presented not just for calendar years but also trial age which provides a more comprehensive representation of development of pest populations after pasture renewal. All sites had been in pasture prior to trial establishment and all were cultivated before new pastures were sown (Chapman et al. 2018a). All were grazed by cattle.

A binomial generalised linear mixed model in Genstat Version 18 was used to determine differences in the presence of individual pest species within each year of the trial and a repeated measures analysis provided an overall representation of presence during the 4 years.

Pest prevalence

Trial location was a major factor in the presence of three pests; black beetle (*Heteronychus arator*) was only present in the Waikato trial (although their range now extends to coastal Manawatu and similarly to Hawke's Bay on the east coast); porina (*Wiseana* spp.) occurred in the two South Island sites but not in the North Island, whereas whitefringed weevil (WFW, *Naupactus leucoloma*) was present only in the North Island sites although both species occur in both Islands. Of the other pests, grass grub was largely absent from the Southland site due to soil type rather than climate. Those species considered for the cross-site analysis of presence and the sites at which they occurred are given in Table 1.

Clover root weevil (CRW, *Sitona obsoletus*) and Argentine stem weevil (ASW, *Listronotus bonariensis*) adults are highly mobile and invade new pastures quickly. Both these insects maintained a moderate to high presence at all sites from Year 1 to Year 4 except at the Manawatu site where low rainfall in the January-March period in each of the first 3 years resulted in very low clover growth and, consequently, a low occurrence of CRW (Table 1, Cosgrove et al. 2018a). Porina invaded the plots in Year 1 at the Canterbury site and in Year 2 in Southland. Adult moths deposit their eggs on pasture during flight. The young larvae spend approximately 6 weeks on the surface where they are vulnerable to predation and dry conditions before forming burrows. Irrigation at the Canterbury site would have aided their survival.

The presence of black beetle, WFW, grass grub and root aphid (*Aploneura lentisci*) generally increased year on year. Black beetle, WFW and grass grub incidence peaked in Years 3 and 4 in Waikato. Densities of WFW and grass grub peaked in 2016 in Manawatu and similarly for grass grub in Canterbury (data not shown). In contrast, root aphid presence indicated that it re-establishes readily in pastures, but conditions will then dictate population growth. A similar pattern of reinvasion of grass grub, WFW, CRW and black beetle

Table 1 Pasture pests found in four major agricultural regions of New Zealand (Waikato (WA), Manawatu (MA), Canterbury (CA), Southland (SO)) and their percent presence in core samples taken in each of 4 years and overall mean presence after establishment of the Species Interaction Trials.

Pest	Occurrence across sites	Year 1	Year 2	Year 3	Year 4	All years
Clover root weevil	WA, MA, CA, SO	45	36	46	38	41
Whitefringed weevil	WA, MA	16	50	67	72	51
Argentine stem weevil	WA, MA, CA, SO	54	59	41	25	45
Black beetle	WA	0	20	30	27	20
Root aphid	WA, MA, CA, CO	6	28	35	37	27
Grass grub	WA, MA, CA	7	24	54	64	37
Porina	CA, SO	64	65	47	49	57

has been reported previously for two trials in Waikato (Hardwick 2004; Thom et al. 2014). All of these soil-inhabiting pests were highly likely to have been present in the pastures before sowing of these trials but a period of starvation after herbicide spraying of old pasture together with physical damage caused by cultivation would have reduced their populations to very low levels. Mass dispersal flights of black beetle adults in autumn can result in rapid re-invasion of new pastures although these generally occur when beetle densities are high. The conditions that trigger such flights are relatively uncommon. Unlike black beetle, grass grub is slow to re-invade new pastures as the first batch of eggs is laid close to where they emerge in spring and before the beetles disperse by flying. Flights from surrounding areas lead to establishment of grass grub in new pasture at very low densities and it takes 2 to 4 years for populations to reach damaging levels. Cultivation and insecticide disrupt the pathogens that help regulate grass grub populations which rely on host presence to replenish the inoculum in the soil needed to infect the next generation (East et al. 1986; Popay 1992).

Adult WFW are flightless and therefore slower to re-establish from surrounding pastures than CRW and ASW. Little is known about the dispersal capabilities of root aphids, but these insects can develop wings as adults although this is rarely observed in the field in New Zealand. It is also likely that young aphids, which at times come to the surface, are transported by wind (Popay & Cox 2016).

Effect of clover

In keeping with its host specificity, CRW was much more common in the plus clover treatments than the pure ryegrass swards (Table 2). Likewise, WFW presence was also significantly higher under clover in 3 of the 4 years of sampling and across all 4 years. The preference for presence of clover, however, was much stronger for CRW which had on average a 46% reduced occurrence in the absence of clover over the 4 years of monitoring compared with a 16% reduction for WFW. This suggests WFW also feed on ryegrass roots to a much greater extent than CRW, an effect most likely related to the requirement of 1st instar CRW to feed on nodules. In general, WFW is considered to be a highly polyphagous pest and has been recorded feeding on 385 different plant species (Bragard et al. 2020).

Grass grub occurrence also tended to be greater where clover was present with a significant 12% difference in favour of clover in Year 4 (data not shown) and an 8% difference over all 4 years (Table 2). In Waikato, where grass grub was most common, populations were significantly higher under clover for each sampling occasion between 2014 and 2016. Their presence was 19% greater with clover than without clover in Year 4, which was reflected in a significant difference in density of 28/m^2 (175/m^2 with clover, 147/m^2 minus clover). Irrespective, both densities were high enough to cause significant damage (Ferguson et al. 2018). Given a choice when in close proximity, grass grub larvae will aggregate under white clover rather than ryegrass and populations in the field reach higher densities under pure clover swards (Kain & Atkinson 1977). A summer peak in clover presence in pasture in our trials would have provided good nutrition to early-stage grass grub larvae but levels were lower in autumn (Chapman et al. 2018c) when third instar grass grub were causing the most damage. Thus, despite showing a preference for clover, it appears that development of damaging populations in autumn is not affected unduly by an absence of clover.

Three pests, black beetle, ASW and root aphid, are not known to utilise clover as hosts. Black beetle occurred more frequently where clover was absent with a significant difference in Year 4 and over the 3 years it was present in the Waikato trial (Table 2). Given its relatively low presence, this suggests that black beetle actively avoided areas where clover is present during

Table 2 Impact of clover on mean percent presence of pests in pasture based on a repeat measures binomial analysis over all years using a generalised linear model.

Pest	No. of years[1]	Sites[2]	Plus clover	Minus clover	SED	P-value
Clover root weevil	4	4	60.6	14.8	2.69	<0.001
Whitefringed weevil	4	2	58.1	42.0	3.74	<0.001
Argentine stem weevil	4	4	40.6	47.1	2.49	0.009
Black beetle	3	1	11.9	23.3	5.43	0.008
Root aphid	4	4	14.1	14.2	2.49	0.586
Grass grub	4	3	28.5	20.3	3.63	0.008
Porina	3	2	53.6	60.4	3.12	0.053

[1] Number of years insect was found in samples
[2] Number of sites insect was found at (see Table 1 for sites)

oviposition. Similarly, ASW presence in pure grass swards was significantly greater than in mixed swards, with a significant difference in Year 1 and over all 4 years combined. Unlike black beetle and ASW, the presence of both root aphid and porina showed little response to the clover treatments for each year of sampling and across all years; however there was some indication (P=0.053) that porina presence was slightly higher where clover was absent. At the Southland site, porina densities in 2016, when populations peaked, were significantly higher in the minus clover than the plus clover treatments (20/m^2 cf. 11/m^2; SED 2.8; P=0.010). Previous research with porina (*W. cervinata*) showed that weight gain was equal when larvae were fed clover or a hybrid ryegrass for 1 month (Farrell et al. 1974) and more recently Atijegbe et al. (2020) found that a sole diet of either clover or the hybrid ryegrass supported development to adults. In reality, however, both plant species are utilised in a pasture and the mixed diet has been found to increase growth of this insect relative to larvae fed a sole diet of either species (CM Ferguson unpublished data).

Effect of nitrogen

In these trials, the amount of N had far less influence on the presence of insect pests than did the presence of clover (Table 3). Of the 4 years of sampling, low N significantly favoured CRW presence in 3 of those years, grass grub in each of 2 years and WFW in 1 of the 4 years, while the repeat measures analysis showed that overall presence of each of these species was higher in the low N treatments. Conversely, root aphids were found more frequently in the high N treatments in Years 2 and 3, but there was only a weakly significant effect over all 4 years. The effect of high N on root aphids was previously reported in the Manawatu trial (Cosgrove et al. 2018a) and is implicated in the occurrence of very high populations recorded in a field trial in Ballarat, Australia (Popay et al. 2021). Unlike the other pests here, aphids feed on phloem sap which is rich in amino acids that are the main transportable form of N within the plant (Dinant et al. 2010).

Although no effect of N on porina presence was found for the combined analysis of the Southland and Canterbury sites, the density of porina over the 4 years in the Canterbury trial was significantly higher under the low N than under the high N (Log porina/m^2 Low N -16.7, High N -37.4; SED 6.69; P=0.009). A similar effect was not found in Southland.

Nitrogen applied in spring reduces ryegrass content and has been shown to increase ASW larval damage whereas autumn applications had no effect (Hunt et al. 1988). Despite this, evidence for an effect of N on ASW in our trials was weak, with only one of the four annual samplings giving a difference approaching significance in favour of high N (P=0.054). In the first 2 years, however, there were significant clover × N treatment interactions (Year 1 P=0.006, Year 2 P=0.046) whereby ASW presence was lower in low N/plus clover treatments than in low N/minus clover. Surprisingly the low N/minus clover treatments had similar numbers to the high N treatments, suggesting that presence of clover has a greater effect on this insect than N *per se*. Furthermore, the presence of clover tended to increase perennial ryegrass tiller density under low N, which runs counter to the theory that resource availability (i.e., amount of ryegrass) was a major factor in the presence of this insect in these trials. This, too, indicates that the presence of clover has a negative influence on ASW. Further research to investigate the reasons for such interactions would increase our understanding of factors affecting this major insect pest.

Effect of cultivar

The only effect of cultivar that did not relate to differences in endophyte was a significantly lower presence of grass grub in the two tetraploid cultivars Base and Bealey, compared with all the diploid cultivars in Year 4 of the all-sites analysis (% presence Year 4: Base 69.1, Bealey 63.1, range for diploid cultivars 83.7-

Table 3 Categorisation of pasture pests according to whether they have a narrow (Specialist) or a broad (Generalist) host range, their preference for feeding on white clover or ryegrass in pasture (see Table 2), and the impact of nitrogen (N) on percent presence.

Pest	Feeding type	Preference	Low N	High N	SED	P-value
Clover root weevil	Specialist	Clover	38.9	29.6	2.55	<0.001
Whitefringed weevil	Generalist	Clover	53.6	46.5	3.69	0.044
Argentine stem weevil	Specialist	Ryegrass	40.5	47.1	2.49	0.011
Black beetle	Specialist	Ryegrass	16.4	17.7	5.43	0.788
Root aphid	Specialist	Ryegrass	11.0	18.0	2.51	0.054
Grass grub	Generalist	Clover	30.6	18.8	3.70	0.010
Porina	Generalist	Either	55.7	58.4	3.12	0.458

90.2; SED 7.06; P=0.012). The effect of the tetraploids on presence of grass grub was also reflected in low densities at the Manawatu and Waikato sites, although AberMagic AR1 also had low densities. This is thought to be the first observation of a suppressive ploidy effect on this insect. The reasons for the lower density and presence in the tetraploid cultivars is unknown but warrants further investigation.

Can we reduce the impact of pasture pests?

The effects of clover and N on our pasture pests are as diverse as the pests inhabiting our pastures, ranging between a strong preference of CRW for the presence of clover in a low N environment to no discernible preference of porina for clover presence or either low or high N. Those pests that had an increased presence with clover (CRW, WFW and grass grub) all preferred a low N environment. In contrast to this, of the three pests that utilise ryegrass and not clover as a host, two, ASW and root aphid, had a higher presence in high than in low N treatments and, perhaps more surprisingly, ASW and black beetle had a significantly reduced occurrence in the presence of clover. Canterbury was the only site where ryegrass tiller densities were lower in mixed pastures than in monocultures but, even then, ryegrass availability would not have limited the occurrence of either pest (Goldson et al. 1998; Chapman et al. 2018c; Cosgrove et al. 2018a; Lee et al. 2018; Stevens et al. 2018). Further investigation is needed to understand this apparent dichotomy.

Utilising the information from this investigation for better management of our pests provides some challenges. Added to this there are clear environmental goals around reducing high N inputs and once again maximising use of clover to naturally fix N to the benefit of the ryegrass component (Chapman et al. 2017, 2018c). Reducing the amount of N applied and increasing clover content of pastures should decrease the presence of ASW, root aphid and black beetle, with the advantage of also being better for the environment. Conversely, the pests that may become more troublesome with an increased use of clover are CRW, grass grub and WFW. Whitefringed weevil appears to be less destructive to clover than CRW while the latter is currently controlled largely by the parasitoid *Microctonus aethiopoides*, although it still reduces on-farm production (Ferguson et al. 2018).

Grass grub, however, remains our most destructive pest, with pasture renewal, drought and insecticide use major factors in reducing the effectiveness of natural pathogens to regulate populations (Popay 1992; Ferguson et al. 2018). Even though the occurrence of clover significantly increases the presence and populations of grass grub, eliminating clover and relying on high N to fill the gap is not an option from an environmental point of view. Moreover, the population reduction in Year 4 under the high N treatment without clover was 13% and with clover was 10%, neither of which is likely to make a significant difference to pasture damage.

Further research

Two new pieces of information arising from this study warrant further investigation. The first, is the reduced numbers and presence of two major pests, ASW and black beetle, in the presence of clover. Second, is the unexpected reduction in presence of grass grub for the two tetraploid cultivars. For both, understanding the mechanisms behind these effects may enable their use for more effective management of these pests.

The increasing presence of grass grub after pasture renewal suggests that conservation and augmentation of its natural enemies could improve pasture resilience. Using less invasive techniques than cultivation for pasture establishment, possibly increasing pasture diversity (although there are conflicting views about this e.g., Gerard et al. 2018; Goldson et al. 2020) and not using pesticide to control pest outbreaks, will also help to retain natural enemies. Although going through a cropping cycle is an option, this too all but eliminates entomopathogens. To address these issues, further development of biopesticides together with smart technologies to enable detection and mapping of soil pest populations that will allow targeted and economically viable application of control strategies, including biopesticides, is needed (Mansfield et al. 2020).

We can also make better use of plant species that are more tolerant or resistant to pests. For example, red clover is less preferred than white clover by CRW, although this depends on the cultivar (Ferguson et al. 2016), with 'Grasslands Relish' showing particular promise (Gerard et al. 2020). Other grass species such as tall fescue can be very productive and are more tolerant of grass grub than ryegrass (Kain et al. 1979).

Maintaining a resilient pasture under environmental constraints after pasture renewal requires a whole-system approach that takes account of the potential for insect invasions and its consequences. A coherent management programme that uses all available options to reduce the impact of insect pests is possible but achieving that requires further research,

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