ITALIAN RYEGRASS DOES IT BETTER: EFFECT OF *LOLIUM MULTIFLORUM* ON ARGENTINE STEM WEEVIL PARASITISM BY *MICROCTONUS HYPERODAE*

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Introduction

Until recently there was confidence that the impact of the Argentine stem weevil (ASW; *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae)) on *Lolium* sp.(ryegrass)-based pasture grasses had declined as a consequence of introducing a biocontrol agent, the parasitoid wasp *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) and the development and adoption of novel ryegrass endophytes that protect plants from ASW attack (Goldson et al., 2014a; Goldson et al., 2014b; 2015). However, there is now mounting evidence that the effectiveness of *M. hyperodae* as a biocontrol agent may have decreased. This in part has been based on reports of the reappearance of notable ASW damage to pasture (e.g. Popay et al., 2011). Moreover, a meta-analysis of historical datasets of winter parasitism rates (that tend to be stable due to absences of both weevil population fluctuations and parasitoid oviposition activity) has shown that parasitism rates have declined by more than 50% in *Lolium*-based pastures since the parasitoid's initial establishment and stabilisation in the first six years following its introduction in 1990 (e.g. Goldson et al., 2014a; Goldson et al., 2014b).

This decrease in parasitism could be attributable to weevil resistance arising from continuous and high parasitoid selection pressure over the last c. 20 years as was suggested recently by Goldson et al. (2015). This could conceivably have arisen because the parasitoid undergoes parthenogenetic thelytokous reproduction whereas the weevil reproduces sexually resulting in what is sometimes described as an 'unequal evolutionary arms-race'. Similar genetically-based resistance, has previously been shown by Pascoal et al. (2014) in two distinct populations of field crickets (*Teleogryllus oceanicus*) on the Hawaiian islands where the crickets stopped stridulating (after c. 24 generations) thereby becoming less susceptible to parasitism by the fly (*Ormia ochracea*). ASW has undergone c. 50 generations since the first releases of *M. hyperodae* suggesting the possibility that such resistance could have developed in this case. Goldson et al. (2015) have recently observed that field parasitism of ASW by *M. hyperodae* was significantly higher in tetraploid *L. multiflorum* paddocks than in diploid *L. perenne* counterparts. This may indicate that other factors, such as plant structure could be aiding the weevils to avoid parasitism and therefore reducing biocontrol efficacy. To explore the implications of this further, this paper describes a systematic laboratory study to determine whether similar plant-mediated differences in parasitism occur in the controlled environment of cages.

Methods

The grasses used in this study were those commonly used in improved New Zealand pastures and were similar to those studied in the field by Goldson et al. (2015). These were 'diploid' *L. perenne*, 'tetraploid' *L. multiflorum* and a 'hybrid' *L. perenne* × *L. multiflorum* cross. All grasses were endophyte free.

All experimental work was conducted in ambient laboratory temperatures (23 ± 2˚C) under a 16:8 L:D photoperiod. ASW adults were collected from mid-Canterbury ryegrass pastures between 11 January 2016 and 22 January 2016. To ensure that parasitoid free weevils were used the ASW were caged between 28 and 55 days at 23˚C (± 2˚C) under a 16:8 L:D photoperiod allowing any parasitoids present to develop to pupae at which point they emerge from their weevil hosts killing them in the process. These pupae were reared to adult and along with the remaining unparasitised weevils used in the experiment.

The experiment used 305 mm × 205 mm × 130 mm deep translucent plastic cages with gauze lids. There were four replicates of the 'diploid', 'tetraploid' and 'hybrid' grass treatments. All cages were stocked with 23 ASW and two parasitoids. Each cage contained one of the grass treatments in the form of two 25 mm wide clumps of grass seedlings with 150 mm long foliage, each with their moistened roots sealed in small polythene bags. This resulted in at least 40 grass tillers per box. Each treatment was split into two sub-treatments, in separate cages, whereby the grasses were positioned either horizontally or vertically; thus the cages were respectively positioned either standing upright on one end, or lying flat. There was also a control treatment comprising four cages (also split into the two sub-treatments) containing 23 *L. bonariensis* and two *M. hyperodae* along with two water-soaked dental wicks to maintain humidity. The treatments were placed randomly in the laboratory. Parasitoids were removed from all cages after 48 hours in order to avoid possible superparasitism (e.g. Goldson et al., 1998). After five days the weevils were dissected to assess parasitism rates. To test for statistical significance in parasitism rates between treatments (including the control cages), non-parametric random permutation tests (n runs = 10000) were run for a one-way analysis of variance (ANOVA) via post-hoc Tukey's HSD pairwise permutation tests using R 3.2.1 (R Development Core Team, 2014) and the package "*lmPem*" (Wheeler, 2010).

Results

Horizontal and vertical treatments combined

The rate of parasitism in the presence of tetraploid grass (75 ± 4%) was significantly higher than in either of the other grass treatments (P < 0.001). There was no significant difference in parasitism rates between cages containing diploid grass (46 ± 5%) and hybrid grass (52 ± 4%; P = 0.8). Parasitism rates in the control cages was 33 ± 7% and was significantly less than that found in cages containing grass (P < 0.01).

Horizontal versus vertical treatments

Horizontal versus vertical positioning of grass bouquets within the cages resulted

in no significant differences in the rates of *L. bonariensis* parasitism by *M. hyperodae* for any of the grass treatments (tetraploid grass, P = 0.8; diploid grass P = 0.1 and hybrid grass = 0.8 respectively).
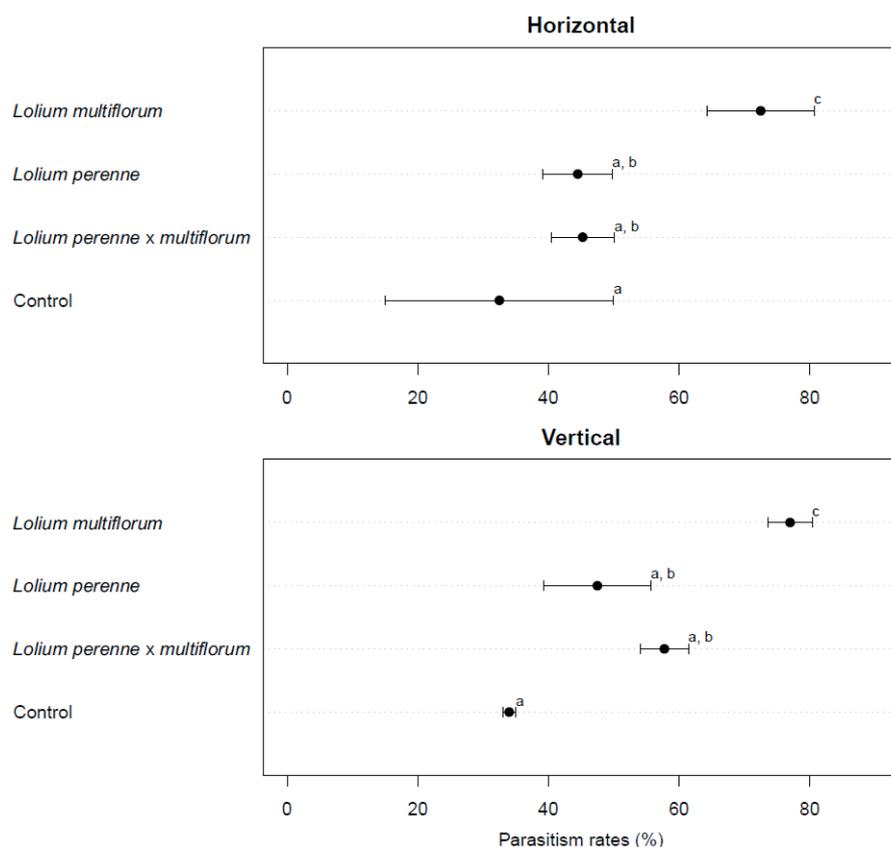
Horizontal treatments

In the horizontal sub-treatments, the rate of parasitism that occurred in the presence of tetraploid grass (73 ± 8%) was significantly higher (P < 0.001) than in either of the other horizontal sub-treatments (Figure 1). There was no significant difference in parasitism rates between cages containing diploid (45 ± 5%) and hybrid grasses (45 ± 5%, P = 0.08; Figure 1). Parasitism in the empty controls in the horizontal subtreatment cages

was 33 ± 18% which was not significantly different from the hybrid and diploid treatments but was significantly different to the tetraploid grass (P < 0.04; Figure 1).

Figure 1.

Cleveland dotplot for *Microctonus hyperodae* mean parasitism rates (%) as measured in *Listronotus bonariensis* in cages containing tetraploid *L. multiflorum*, diploid *L. perenne*, hybrid *L. perenne × L. multiflorum* and in cages containing no *Lolium* spp. The horizontal and vertical orientations (i.e. subtreatments) are shown here. Error bars represent S.E.M.. Means with different letters were significantly different in pairwise comparisons.

Vertical treatments

In the vertical sub-treatments, the rate of parasitism in the presence of tetraploid grass (77 ± 3%) was significantly higher (P < 0.001) than in either of the other upright grass treatments (Figure 1). Again, there was no significant difference in parasitism rates between the cages containing the diploid (48 ± 8%) and the hybrid grasses (58 ± 4%, P = 0.08; Figure 1). Parasitism in the empty vertical control was 34 ± 1% and was not significantly different to the hybrid and diploid grasses (P = 0.5; Figure 1).

Discussion

Grass types and parasitism rates

The combined data from the horizontal and vertical sub-treatments of all three grass treatments showed parasitism rates to be very similar to those observed in the field (Goldson et al., 2015). This is surprising given the obvious environmental differences between field conditions and those of laboratory cages (e.g. no soil or detritus). The avoidance of parasitism by the weevils abandoning the foliage in the presence of the parasitoid as reported by Gerard (2000) seems unlikely here given that the grass leaves in the horizontal treatment were fanned out across the floor of the cage. Also all grass treatments were tightly bundled at their stem bases thereby probably providing limited scope for the weevils to 'hide' from the parasitoids.

In general, the results of this study support the contention of Goldson et al. (2015) that if selection pressure has led to an enhancement of some kind of parasitoid-avoiding behaviour that is influenced by plant architecture, then such evolution would most likely have occurred in the country's extensive diploid pastures rather than in the less common tetraploid *L. multiflorum* pastures (B.R. Belgrave, Grasslanz Technology Ltd, pers. comm.). The possibility that resistance is dependent on plant type would seem to be unique in the literature.

Plant orientation effects

Horizontal versus vertical positioning of grass within the cages resulted in no significant differences in the rates of parasitism of *L. bonariensis* across all of the grass treatments. This indicates that, at least in the absence of soil or detritus, the orientation of plant

Horizontal

Vertical

Parasitism rates (%)

material does not affect parasitism rates. This is somewhat contrary to Phillips (2002) observation that plant orientation in a cage (albeit with soil and detritus) may influence parasitoid efficacy. The results of this study also point to the possible incorrectness of the contention of Goldson et al. (2015) who suggested that the higher levels of parasitism in the tetraploid *L. multiflorum* could have resulted from a difference in the architecture of the tetraploid versus hybrid and diploid plants. This hypothesis would seem to be logical in the case of the plants being placed vertically. However, it can be argued that horizontal plant placement is such a gross departure from natural vertical growth that this would have overridden any relatively subtle differences arising from *Lolium* spp. plant morphology; yet there were no differences in the levels of parasitism. Plant architecture thus looks to be unlikely as a mechanism enhancing parasitism resistance. Thus, what caused the observed patterns of parasitism remains obscure.

It is also of interest that parasitism rates in grass-free control cages, while usually lower than in the cages with grass present, still showed substantial attack rates indicating that *L. bonariensis* is susceptible to parasitism even when not feeding.

In summary it has been confirmed that different patterns of parasitism associated with different *Lolium* spp. and ploidy observed in the field also occurred in this laboratory experiment and that plant architecture is unlikely is play a significant role in the apparent evolution of weevil resistance to the parasitoid. At the same time, it has been demonstrated in the laboratory that diploid *L. perenne* (cv. Grasslands Samson) and the hybrid *L. perenne × L. multiflorum* (cv. Grasslands Manawa) no longer support the levels of attack that were found in caging experiments in the 1990s (Goldson and Tomasetto, 2016). This is consistent with the contention that the weevil has evolved resistance to the parasitoid.

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