Snails in Bales: The likelihood of *Galba truncatula* being picked up and surviving baling of round bales for silage

Dr Neil MacKintosh, Kate Heduan, Rhun Fychan, John Davies. IBERS, Aberystwyth University
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Executive summary

The aim of this research project was to determine if there is a possibility for the intermediate host for *Fasciola hepatica* (liver fluke), *Galba truncatula* (mud snail), to be incorporated into round silage bales at different stages in the silage production. The project was split into two parts:

1) Determining whether the mimic snails (gelatine capsules filled with metal filings to a typical weight of snails of similar shell length) behaved in a similar way to snails in a grass sward under typical field preparation for silage making
2) Using a combination of actual snails and mimic snails to determine whether there was a possibility that *G. truncatula* is able to survive incorporation into a round bale of silage

The findings of the current study showed that "mimic snails", which were gelatine capsules of 8mm length filled with metal filings to a typical weight of a similar sized *G. truncatula*, behaved in a similar manner when mowing or raking. Through the use of these mimic snails and real *G. truncatula*, it has been shown in this project, that it is possible for *G. truncatula* snails to be picked up by low and normal height baler tines and also to then be included undamaged within the bale (n = 13/135 low; 1/135 normal) ($p < .05$), but the risk is low of the snail being raked across the field and into the row, based on our observations of the disturbance of the mimic snails by rake tines at low or normal height. Although it was found possible for snails to survive bale formation, snails are more likely to be damaged if baled into the outer-point of the bale's radius ($p < .05$) than the inner or centre-points of the radius. These results show that snails can survive incorporation into a bale and this could happen if a row for pick up is lying over a snail habitat and the baler tines are low at this point in the bale run. However, this project found no indication that the rake will pull snails not on the swath into the row even when tines were too low. This project was unable to look at the possibility of parasite survival through use of the snail as a protective vessel during the initial fermentation process, and it remains to be seen whether *F. hepatica* can survive ensiling through incorporation of its intermediate host.

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Therefore, the advice to industry from this research, is ideally not to cut grass in suspected snail habitats, but if this is unavoidable, to pull the cut swath out of that habitat to form rows for baler pick up outside of the suspected snail habitat to ensure the least risk of *G. truncatula* becoming incorporated into the bale. As these habitats will often be damp and not suitable for heavy machinery (i.e due to tyre rutting) this advice may already be followed but any swath cut around the edges of such habitats should be raked out of the habitat for baling to minimise risk of incorporating the snail into the bale.

Introduction

Any factors that could affect the quality and biosecurity of baled silage produced globally could have long-ranging effects on the livestock consuming the end product and thus impacting on the efficiency of production. Fasciolosis (liver fluke) was estimated in 2005 to cost the agricultural sector up to £300 million per annum in production losses in the UK (Bennet & Ijpelaar, 2005) and this number is thought to have increased in recent years due to resistance to drug treatments, climate change and more frequent animal movements (McCann *et al*., 2010; Williams *et al*., 2014). There has been previous research on silage being a possible source for infection by *Fasciola hepatica*, however, there are still gaps in our knowledge. Previous research does appear to show that silage which has under gone the correct fermentation pattern is of limited risk to livestock when looking at the metacercariae stage which is regarded as the infective stage to livestock (Boray & Enigk, 1964; Ollerenshaw, 1971; Smith, 1982). However although the stages within the intermediate host are not infective to the vertebrate host (livestock), it is known that certain conditions may cause rapid shedding of the parasite from the host into the surrounding environment (Ollerenshaw 1971). We therefore hypothesise that incorporation of the snail into silage may provide a vehicle for the parasite to survive long enough to be shed from the snail onto the surrounding silage and remain infective for long enough to infect the livestock that consume the silage.

Methods

1. Mimic snail preparation

Fifteen *G. truncatula* snails were previously weighed and their shell lengths measured to find the predicted weight of a snail at 8 mm. This was calculated by producing a correlation graph and finding the line of best fit (y = mx + c). At a length of 8 mm, snails were predicted to weigh 0.065g. Yellow gelatine capsules measuring 8 mm (Dr T & T Health UK Ltd, Corby, UK) were filled with iron filings (Fisher Scientific UK Ltd) using a manual capsule filling machine, model CN100 (Dr T & T Health UK Ltd, Corby, UK) to a total weight of 0.065g.

2. Testing mimic behaviour against live snails

To indicate whether mimic snails would be disrupted to a similar level as live *G. truncatula* or similar sized mollusc, they were tested for their effectiveness as mimics of real-life snails in the trials. This involved several trials in small plots to determine if the proposed mimics, gelatine capsules of the same weight and length of an 8 mm *G. truncatula* snail, would suitably represent a live snail for use in other trials of this project. In a series of grass areas (referred to as plots) (1.25 m x 1 m) of unmown area of principally grass sward, mimics were placed in an "X" formation (see figure 1) inside a 45 cm x 45 cm area within the plot. The following comparisons were performed with a minimum of n=3 by plot:

- Capsule degradation over 3 hours
- Suitability of different types of original location marker as a way of measuring how far a mimic or snail has been moved by a treatment (golf tee, lollipop sticks, match sticks were compared)
- Capsule with or without petroleum jelly as a mimic for snail mucous
- Behaviour of mimic compared to live snails of similar size during mechanical field work
- Behaviour of mimic compared to live snails during raking

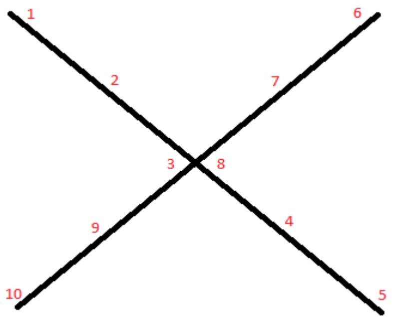

Figure 1. Diagram showing layout of the ten capsules in the "x" formation. Approximately 10 cm between each respective capsule.

3. Rowing up

Six plots of approximately 2.15 m x 6 m were marked out in the meadow (ryegrass approx. 80 %; white clover approx. 20 %; some thistles and docks) after the grass was mown the day before. Each row was approximately 17 m in length containing two plots per row, with a 4 m gap between the parallel rows (two plots per row, three rows, giving six plots in total) (figure 2). A representative grass sample was collected from the sward for further analysis. See figure 2 for layout of quadrats and figure 3 for mimic snail layout within the quadrats. The mimic snails and respective wooden markers were placed under the row by carefully lifting the grass up and replacing once the mimic snails were in place on top of the wooden markers, which were pushed fully into the ground until the top of the golf tee was level with the soil surface. The centre mimic also had an RFID tag placed nearby into the ground. Once all (n = 180) mimic snails were in place, the grass was left to wilt for a minimum of 24 hours. After 24 hours the rows were raked left to right (Kuhn GA 7501, Kuhn farm machinery UK LTD, Telford, UK) gathering it into rows of about 2 m wide (3.2 mph; 3500 RPM). Plots 1, 4 and 5 were raked at normal tine height with intermittent tine contact to the ground. Plots 2, 3 and 6 raked at a low height where the tines continuously scratched the surface of the ground. The grass was always raked in the same direction; left to right. After raking, mimic snail movement and damage was recorded (distance from centre of marker to centre of mimic) and any damage was scored 0 – 5, with 0 being no damage and 5 being completely crushed (see table 1).

Table 1: Damage scores and their criteria for ranking of mimic snails post-rowing up.

DAMAGE LEVEL	DESCRIPTION
0	no damage
1	one small dent/crack
2	one dent & crack
3	more than one dent(s) and/or crack(s)
4	split(s) in capsule (metal leaked out)
5	completely crushed

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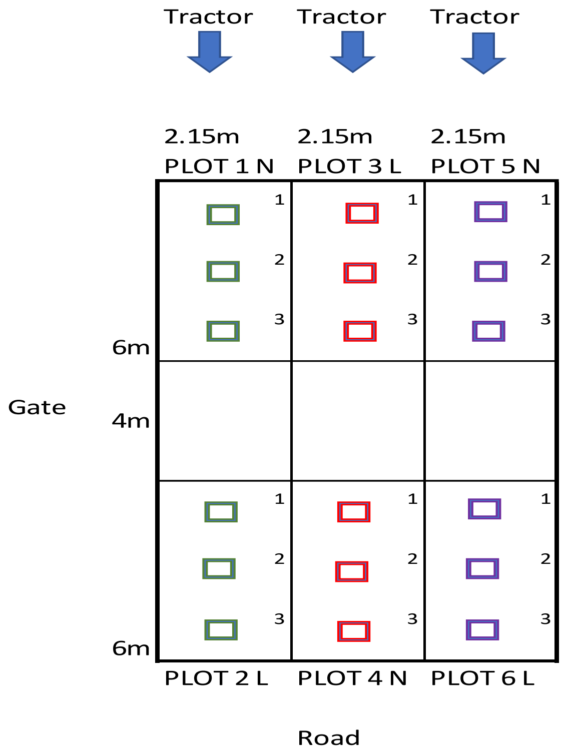

Figure 2: The layout and dimensions of the 6 rowing up plots is displayed. The coloured squares represent the quadrats and tine height is denoted by "N" = normal and "L" = low.

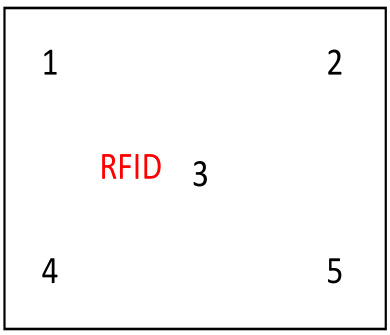

Figure 3: Layout of the mimic snails inside the quadrat area where numbers 1 – 5 represent the position of the mimics.

4. Baler pick up

This involved recording the number of mimic snails that are taken into the baler and incorporated into the bale or distance moved from the marker. Level of damage for each mimic snail was also recorded. Rows were prepared as for rowing up. Mimic snails (n = 360) were labelled and split accordingly into groups of 15 per transect.

Three transects per plot with 15 capsules per transect were laid out (Image 1; Figure 4). Grass samples were collected from the plots for DM analysis. Transects were built to approximately 3 cm below the height of the aftermath to help with creating a low tine height. The plots were baled in the following pattern: 3, 6, 7 = normal height and 4, 5, 8 = low tine height. Plots 1, 2 and 3 were test plots and data was not recorded. The grass from the adjacent row was carefully raked in place over the transects after all mimic snails were in place. Once the baler passed over the first plot, the transects were checked for movement of the mimic snails using a tape measure, and the bale of grass was taken from the baler and checked for mimics by shaking the grass out onto a tarpaulin.

Image 1. A picture taken of a transect of 15 mimic snails on a raised sand mound.

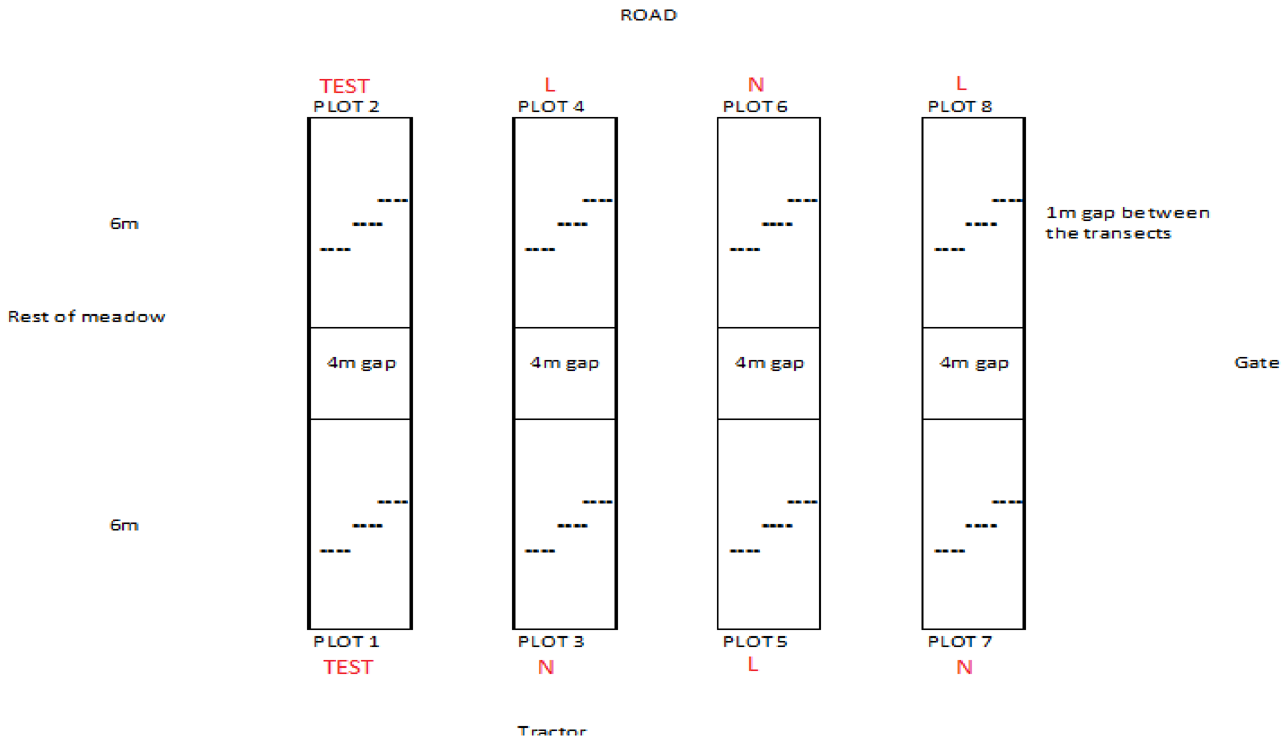

Figure 4: Layout of the 8 baler pick-up plots is shown. The dotted lines in each plot represent the transects of mimic snails.

Three rows of cut and raked grass were prepared, with two rows measuring 37 m total length and the other 35 m. Each row was determined to be long enough to form a whole bale based on density of the sward. Approximately 150 *G. truncatula* snails were collected the day prior to this trial and stored in falcon tubes in the lab with some of the substrate in which they were found. Ninety of these snails (average of 4.26 mm in length, min. = 3 mm, max. = 6 mm) were randomly separated into groups of 10 and carefully placed inside 9 cryovac bags (approx. 15 x 15 cm). The bags were then labelled 1 – 9. As each row was picked up three bags were dropped in numerical order in the path of the baler just in front of the tines to ensure its uptake into the machine. The first bag was dropped 5 m along from the start of the row, the second bag towards the middle of the row (17 m) and third near the end of the row (33 m).

After each bale was fully formed, it was dropped out of the machine and unrolled. When a bag was spotted, a metre stick was used to measure the distance from the centre of the bale (radius) at which it was found. The snails from the bags were stored at 4 ℃ until examined under 10 x magnification to identify any damage to the shells. Each snail was damage scored from 0 – 5, with 0 being no damage and 5 being completely crushed (see table 2 of scale).

Table 2: Damage scores and their criteria for ranking of mimic snails post-baling.

DAMAGE LEVEL	DESCRIPTION
0	no damage
1	one dent/crack
2	2+ dents/cracks
3	shell splintered, mantle exposed
4	partly crushed
5	completely crushed

6. Statistical analysis

All tests were performed in SPSS version 25 and at a $p < 0.05$ significance level.
For distance moved an independent samples t-test was conducted or a one-way ANOVA. For damage scores either Mann-Whitney U and/or Kruskal-Wallis test were conducted.

Results / Discussion

1 Mimic snails vs. live snails

The preliminary trial to test the suitability of gelatine capsules as mimic snails showed no issues with deterioration of the capsules after three hours.
Distance moved by mimics post-mowing did not significantly differ between petroleum jelly-coated and non-coated groups when mowed over by the lawnmower ($t = -.651$, $p = .248$). There was also no significant difference in movement observed between coated and non-coated mimics after being raked over with a handheld garden rake ($t = -.105$, $p = .803$). The comparison of movement of non-coated mimics, coated mimics and live snails post-mowing found that there was no significant difference between the variances of means in any of the groups ($F = .337$, $p = .715$). Also no significant difference was found with post-raking movement between the three groups ($F = 2.017$, $p = .135$).

Golf tees pushed into the soil until level with the soil surface, were found to be the most suitable markers to use. It was therefore concluded that petroleum jelly was not required and that golf tees would be used as the markers.

2 Rowing up of mimic snails

No significant difference was found in distance moved of mimic snails between normal (mean: 0.378 cm) and low tine-height (mean: 0.867 cm) groups after raking (t = -.717, p = .091), but the mean distance moved by mimics was greater in the low-raked plots than the normal-raked plots (low = 0.867 cm, normal = 0.378 cm).
Plus no significant difference in number of mimics damaged between the two tine height groups of mimic snails (p = .400), but the mean number of damaged mimics was higher in the low-raked plots (n = 16) than the normal-raked plots (n = 13).

Table 3. The number and percentage of mimic snails damaged at each tine height post-rowing with the mechanical rake.

Rake Tine Height	Number damaged	% Damaged
Normal	13/45	28.89
Low	16/45	37.78
Total	29/90	33.33

4.3 Baler pickup of mimic snails

Though there was differences in the means, positioning of the transect to the (left, middle, right) line of the baler's path had no significant effect on distance mimics were moved after baling (F = .948, p = .397).

At plot level, there was no significant difference in mimic snail movement between all of the low-baled plots (n = 9) post-baling (F = .032, p = .969). But there was in the normal-baled plots (n=9) (F = 12.360, p = .000) due to plot two being an outlier, with a mean movement distance of 3.4091 cm +/- 8.05668 cm compared to plot one: 0.5222 cm +/- 1.36080 cm and plot three: 0.1556 cm +/- 0.75244 cm.

No significant difference was found between the low-baled transects (F = .508, p = .848). There was significant difference between the distance mimics were moved with low-baling (n = 119) to that of the normal-baled mimics (n = 134) (F = 66.040, p = .000), with mimic snail average distance for low-baled plots being 12.1387 cm +/- 20.24500 cm and normal-baled plots 1.3470 cm +/- 4.88908 cm. Thirteen mimic snails were baled from the low-height plots and only one from the normal-height groups. This indicates there is a higher chance of snails being picked up into the baler if the baler is set to a lower tine-height than normal, in particular where the tines scratch the surface of the ground (p = .001).

Table 4. Shown below is the number and percentage of mimic snails from each tine-height group that were incorporated into bales.

Baler Tine Height	Number baled	% Baled
Normal	1/135	0.74
Low	13/135	9.63
Total	14/270	5.19

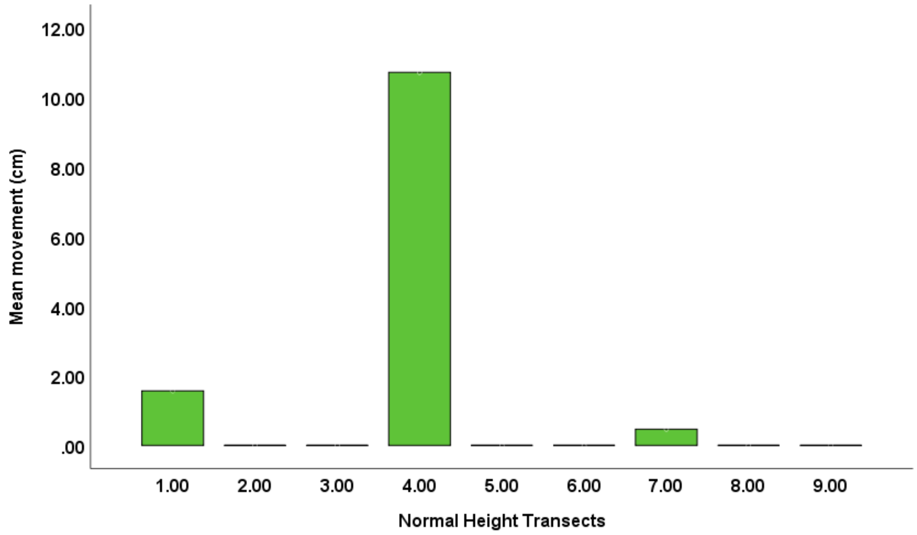

Figure 5. Depicted here is the mean distance moved by mimic snails in the nine transects baled at normal height. Transect number is on the x axis. The greater movement distance that occurred in transects 1,4 and 7 are clearly seen here.

Image 2: Depicted above is a transect with several mimic snails moved post-baling on a low tine height. The furrows in the sand bank show where the baler's tines made contact with the transect.

4 Baler rolling and compaction

The positioning of the bags in each bale had a significant difference on the level of damage seen on the snails (n = 9 bags, n = 10 snails per bag) (H = 36.485, p = < .000)(see figure 6). The differences were significant between the centre vs. outer radius point groups (p = .000) and the mid vs. outer radius (p = .000) groups. There was no significant difference in number of damaged mimics between the centre vs. mid radius groups (p = .607) (see table 5).

Table 5. Below is shown the number and percentage of snails that were damaged at each radius depth.

Radius depth	Number damaged	% Damaged
Outer	28/30	93.33
Mid	11/30	36.67
Centre	10/30	33.33
Total	49/90	54.44

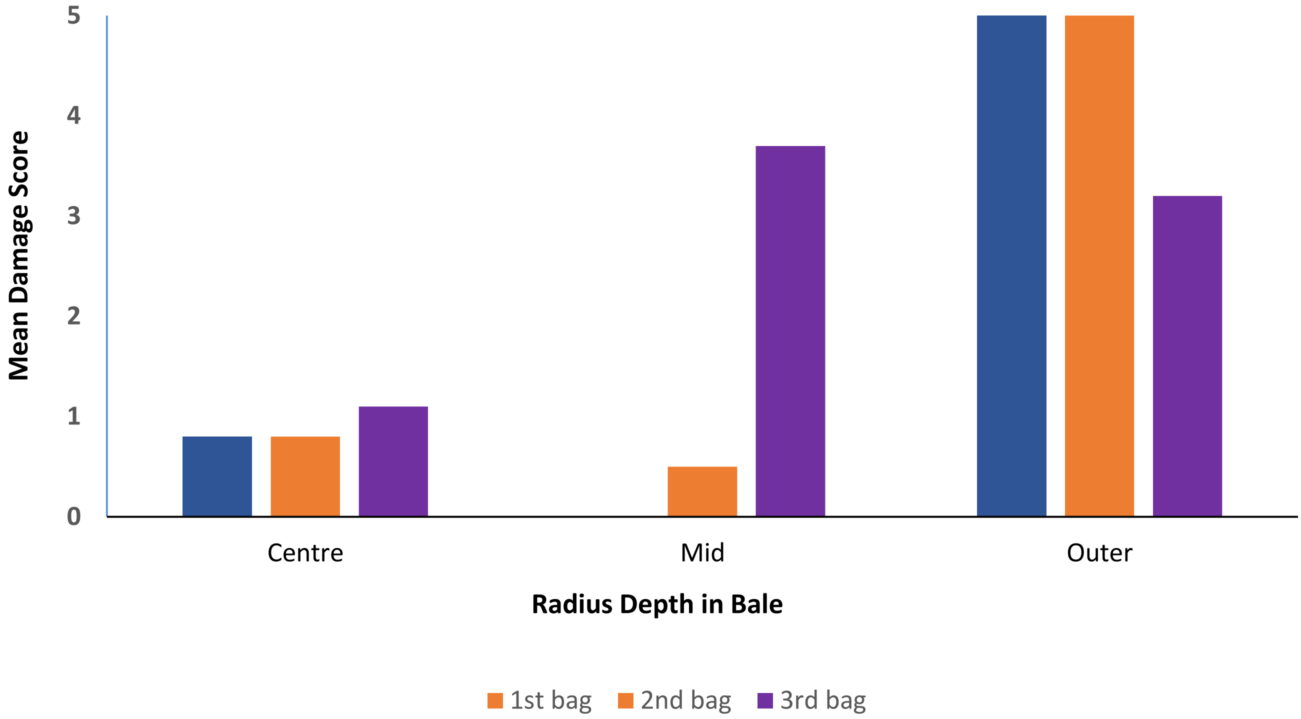

Figure 6: The graph above shows the mean damage score for each bag of snails. Snails received a score from 0-5, where a higher number = increased level of damage. There was no damage to any of the snails in the first bag of the mid-radius bale depth group.

5 Discussion

A study by Laws *et al.,* 2002 found that different types of machinery used in meadows can affect the level of soil disturbance and therefore contamination in the silage especially if tines are set to the wrong height. This was seen in this study by the greater mean disturbance of mimic snails by the rake when set at a low tine height, where the lowered tines caused more damage and moved the mimics from their markers more often, and on average further, than the tines at a normal height. However, this study showed, based on the findings with mimic snails, that *G. truncatula* snails are unlikely to be pulled into a swath, irrespective of height of the tines. The recommended mowing height for ryegrass silage is that there should be at least

5 cm stubble left in the field (NRCS, 2010; AHDB, 2013). The recommendation for rake tine-height collaborates with the mowing height as rake tines should be low enough to scrape all of the cut grass lying on top of the stubble into rows while being high enough to avoid contamination of the crop by scratching soil, stones or manure into the row (AHDB, 2013). In the current study, the low rake tine-height was set up so that the tines scraped the surface of the ground on the gathering side of the rotation, thereby not following the recommendations. It has been found in previous studies on fodder contamination by soil that crops harvested in wet weather and wilted for long periods are more likely to be affected and may result in losses of 2 – 12 % DM (Volac, 2001). Silage contaminated with soil poses a risk to animal feed safety as microbes, fungi and spores could enter the silo via soil particles and produce harmful compounds such as butyric acid and reduce lactic acid fermentation of silage, therefore, good practice is to minimise scratching for these reasons already (Wilkinson, 2005). This combined with the results of this study make it highly unlikely that snails will be raked into the rows if good silage making practices are followed.

Examination of the results for the baler pickup of mimic snails indicates that there is a chance that *G. truncatula* could be picked up by the baler's tines and incorporated into bales if present under the row of wilted grass. The results show that if the baler is set to a low tine-height the likelihood of the mimic snails being moved from their original positions is significantly higher than when set to a normal tine-height as used on farms. It was also determined that a significantly greater number of mimic snails were baled up from the low-baled plots (n = 13) than the normal-baled plots (n = 1), which indicates that setting the balers tines to too low a height where they scratch the surface of the ground increases the risk of the silage being contaminated with *G. truncatula,* Although a low percentage of all mimic snails were incorporated into the bales overall (5.19 %, table 4). As with the raking up of grass into swathes, too low a tine height on silage balers could result in other undesirable contaminants entering the silo, so good silage making practice that ensures correct tine height is also recommended as a way of minimising the risk of snail pick up, if there is no way of avoiding picking up rows that go through snail habitats. However, the results of this trial indicate that the risk of snail pick up into the bale will significantly increase if the baler is set too low or if there is uneven ground under the swathe when passing through the snail habitat, therefore the results suggest that the best way to reduce the risk of picking up *G. truncatula*, is to form the row outside of the habitat.

The results of the compaction of the snail during baling in this trial show that *G. truncatula* snails located in the outer-point of the bale radius experienced the most damage out of the three radius points. The number of damaged snails in outer-point radius bags was significantly higher than those in the centre or mid radius bags. As seen in table 5, 93.33 % of *G. truncatula* snails in the outer bags experienced some damage whereas the mid and centre radius bags of snails had 56.66 % and 60 % less damage than this, respectively. This large increase in number of damaged snails in the outer radius reflects the findings of previous literature where it has been found that the DM density of silage bales made by fixed chamber balers (as used in current study) is lower and less uniform at the central core of the bale (Weinberg & Ashbell, 2003). McEniry *et al.,* 2006 found that a larger proportion of silage in round bales is in contact with the polyethylene barrier of film in comparison to clamp silage, resulting in baled silage being more vulnerable to having a less anaerobic environment especially nearer the outer radius than the centre. Therefore, although the majority of snails were damaged or crushed in the outer radius of the bale, some did survive the baling and the less strict anaerobic conditions of the outer radius segment of the bale may provide sufficient amounts of oxygen to enable the survival of *G. truncatula*. In this research project, *G. truncatula* snails experienced high levels of damage in the outer radius, however two out of the 30 snails did survive with no damage so the chance that snails will survive the rolling process unscathed and persist in the outer layer of silage bales could exist. These findings show that *G. truncatula* has the potential to survive baling and the environment may be survivable during fermentation.

Summary

This study indicates that 8mm sized objects similar in weight to *G.truncatula* on the soil surface are unlikely to be raked into a row, even with tines set so that they continuously scratched the surface of the ground on the gathering side of the rotation. However, the study has also shown that it is possible for these objects, to be picked up by baler tines at the recommended height or a lower setting when on the soil underneath the row of wilted grass. The risk of the snail being picked up could also significantly increase when tines are set too low compared to the recommended height. In addition to this, the study has shown it is possible for the snails to survive the actual baling process, however, the trial was unable to look at whether either *G. truncatula* or *F. hepatica* could survive a 90 day fermentation in round bales.

These findings have identified possible ways of minimising the risk of baling *G. truncatula* and, until it has been confirmed whether the parasite, *F. hepatica* can use the snail as a vehicle or lifeboat to surviving the fermentation to achieve successful infection of livestock, the industry

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may wish to consider the following recommendation for silage making on land containing suitable habitats for *G. truncatula* where possible (in order of least risk of snail pick up):

- Avoid cutting the habitats.
- If the habitat is cut try raking up the grass to form rows outside of the habitat
- If the row for baling passes through the habitat try to ensure tine height is kept normal or slightly higher and avoid soil scratching by the tines.

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