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EVALUATING RESISTANT BRASSICA TRAP CROPS TO MANAGE *HETERODERA SCHACHTII* (SCHMIDT) INFESTATIONS IN EASTERN ENGLAND

Evaluating brassica trap crops for H. schachtii *control in East England*

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Abstract

BACKGROUND:

The beet cyst nematode (BCN), *Heterodera schachtii* Schmidt, is a plant parasitic nematode which causes severe losses to yields of sugar beet. Resistant brassicas (radish and mustard) have been bred to be planted after the harvest of a main crop, such as a cereal, and encourage hatch of BCN juveniles. The resistant plants stimulate hatch of the juveniles but are not suitable hosts. Juveniles are unable to complete their lifecycle and thus populations are lowered. This research aimed to investigate the effectiveness of a range of these brassicas in terms of BCN control when grown in infested fields in Eastern England.

RESULTS:

Experiments were sown using four different radish cultivars, which differed in their resistance to BCN, and one resistant mustard variety. Field experiments were sown in early September in 2016 and 2017. Significant reductions in BCN populations were only found following the resistant mustard and the radish with the greatest resistance level.

CONCLUSIONS:

Further research is needed to understand how best to utilise the brassicas and whether they are economically viable when alternative management options for BCN are available. Time of planting may be crucial to fully achieve their BCN reducing potential.

Keywords

Beet cyst nematode, radish, mustard, nematode management, IPM

1. Introduction

The beet cyst nematode (BCN), *Heterodera schachtii* Schmidt, has been a well-known pest of sugar beet for over 150 years[1]. It can now be found in all major sugar beet producing areas of the world[2] and is a major problem for growers with infestations, especially in years of limited water supply[3]. Yield losses can be severe (30-40% on susceptible cultivars[4]) and have been calculated to be as high as £3.8 million per year in the UK.[5] However, modern varieties of sugar beet with BCN tolerance may be able to overcome the majority of the yield losses[6]. These tolerant varieties still lead to a build-up of BCN populations,[6] which pose a problem for future sugar beet crops in the infested field and increase the chance of the transfer of infested soil to other fields. BCN is also harmful to a wide range of crop species, particularly brassicas such as oilseed rape (*Brassica napus*)[7] and, therefore, alternative methods of reducing populations are important to investigate, especially since chemical control options for BCN control are no longer available[8].

The use of BCN resistant brassicas has become commonplace in Europe. In countries such as Germany, where BCN infestations are widespread, and may exceed 50% of the area cultivated with sugar beet[4], they are a popular option to cultivate. The use of oilseed rape as a trap crop was proposed in the late 19th century by Prof. Julius Kühn.[1] The oilseed rape, which is susceptible to BCN, would be planted to stimulate juvenile emergence and root invasion in the summer prior to sugar beet being planted in the following spring. The seedlings of the oilseed rape would then need to be destroyed before the nematodes could reproduce. Therefore, the use of susceptible trap crops is a risk for the grower, as failure to destroy the crop before a completed lifecycle could exacerbate an infestation and this method was not implemented in the 1800s.[1] However, there are now tools available to advise growers when to destroy OSR volunteer seedlings in Germany to maximise BCN population control.[9] More suitable for BCN control is the use of resistant brassica varieties which offer an option for BCN control with much less risk to the grower. The resistant brassicas stimulate the hatch of juveniles from the cysts in the soil, and whilst they allow for root invasion, they are resistant to the nematode, and prevent the completion of its lifecycle, hence lowering populations within the soil.[10] Resistant varieties of both white mustard (*Sinapis alba*) and oil radish (*Raphanus sativus*) are now available for cultivation as a trap crop and could be incorporated as part of an integrated pest management (IPM) strategy for BCN control.

The use of trap crops for BCN control has been investigated in many locations worldwide and mixed results have been obtained. Research in Germany in the 1990s found reductions in BCN populations using brassica trap crops and improvement to yield of subsequent beet crops[1] and similar results were found by Hafez[11] in the USA. However, Koch et al. [12] found significant reductions in BCN populations using oil radish at only one site out of four also in the USA. Kenter et al.[13] found BCN populations were lowered at twelve out of thirteen sites in Germany where resistant radish variety ‘Adagio’ was planted and the best results were obtained where the cover crop was sown in July rather than August. Hauer et al. [6] found resistant mustard to reduce populations of BCN at five out of eight environments tested between 2013 and 2014, also in Germany. Mustard varieties Luna and Accent and oil radish variety Colonel (the latter two varieties are also evaluated in this paper) have been found to reduce BCN populations at one site in Iran but did not produce significant reductions at a second site.[14] It appears, therefore, that the use of such trap crops is highly variable and further research into their use is necessary, especially as different results are obtained by different researchers in different countries and in different years. Findings from one country cannot simply be expected to work in another country and, likewise, the findings from one variety of trap crop cannot be expected to carry over to another variety.

It is clear that gaps in our understanding of the use of trap crops remain. As their use appears variable between years and countries in which the experiments are conducted, due to different climatic conditions and weather patterns, as well as time of sowing. We therefore decided to conduct two field trials, over two years, to see how a range of commercially available trap crop varieties could reduce BCN populations on infested fields in Eastern England. To do this, on farm field experiments were conducted in fields with known infestations of BCN.

2. Materials and methods

Brassica variety selection

Following a recommendation from a seed merchant five brassica treatments were selected to be sown in a field experiments. Four of these treatments reportedly had some resistance to BCN from bioassays performed on these varieties in Germany[6]. These were one class one radish (with ≥90% resistance to BCN), two class two radishes (70-90% resistance to BCN) and one class two mustard. The final brassica treatment was a tillage radish with no reported resistance to BCN. A fallow treatment was also included in the experimental design. Details of the brassica treatments are listed in table 1.

Table 1 – Descriptions of the varieties of radish and mustard used in the field experiments in Norfolk, England.

Species	Cultivar name	BCN resistance Class †	Seed Rate Kg ha^{-1}	Treatment name
Raphanus sativus	Colonel	1	22	Class 1 Radish
R. sativus	Defender	2	22	Class 2 Radish A
R. sativus	Bokito	2	20	Class 2 Radish B
R. sativus	Early Mino	-	12	Susceptible Radish
Sinapis alba	Accent	2	18	Class 2 Mustard

† Class 1 is stated as having an reproductive factor (*Rf*) of ≤0.1 and class 2 a reproductive factor (*Rf*) of between 0.1 and 0.3 when tested in official laboratory bioassays[6].

Field experiments

Fields with known infestations of BCN were selected in 2016 (Brettenham, Norfolk, 52° 24' 22.6296'' N, 0° 51' 1.4688'' E) and 2017 (Bridgham, Norfolk, 52° 26' 0.1932'' N, 0° 52' 9.5016'' E). Both fields used are of a loamy sand soil type over chalk bedrock.[15] Areas of each field were surveyed for BCN populations on a 50 x 50 m grid spacing comprising 40 soil cores (150 mm deep, 25 mm diameter) and extraction of the cysts. Once areas with the greatest populations had been identified, the field was lightly cultivated using a disc harrow (Simba Xpress 5.5, Simba International Limited, Sleaford, UK) and then the plot layout was marked on the field. Plots were 6x9 m and centred between wheelings in the field. In both years, 24 plots were sampled over the high population areas. Surveying and sampling took place immediately after harvest of the previous crop, which was winter wheat (*Triticum aestivum*) in both years. From each plot, 40 soil cores were taken in a zig-zag pattern, using an AMS EZ-Eject soil probe (AMS Inc. American Falls, ID, USA) (25mm diameter), to a depth of 150 mm to create a bulk sample of each plot. This bulk sample was then sieved through a 4 mm sieve to remove large stones. The soil was then thoroughly mixed together before two 200 ml subsamples were taken. Both were weighed and one was washed using a Wye washer to extract cysts[16]. The other subsample was dried at 105°C for 24 hours to determine moisture content of the soil and thus the dry weight of the washed sample was calculated. Cysts from the sample were counted and then crushed using a Reid aluminium slide.[17] To determine the mean number of eggs per cyst, the crushed cysts were then diluted in 50ml of water inside a measuring cylinder and thoroughly mixed using a glass pipette and pipette controller (Powerpette, VWR International, Radnor, PA, USA). A 1ml subsample was then dispensed into a Fenwick slide[18] and the number of viable eggs and juveniles counted using a stereomicroscope (Leica M80, Leica, Wetzlar, Germany) and tally counter. The population of BCN eggs per gram of dry soil was determined for each plot using the cyst and egg data. The field experiments were established using randomised block designs with the field plots grouped into blocks of similar *Pi* prior to sowing of the brassicas. Each block contained one replicate of each treatment (Table 1). Initial plot populations ranged from 2.1

to 14.8 eggs g^{-1} dry soil in 2016 (mean 7.8) and 2.1 to 24.9 eggs g^{-1} dry soil in 2017 (mean 7.5).

Field experiments were sown on 2 September 2016 and 1 September 2017 using a Horsch Pronto 6 metre seed drill (Horsch Maschinien GmbH, Schwandorf, Germany). The selected varieties of the radish and the mustard were drilled at the seed rates stated in Table 1 following guidance from the seed merchant. There was also a fallow treatment where the drill and tractor passed over the plot but no seed was sown. Soil temperatures were monitored throughout the experiments using a temperature logger (Tinytag Plus 2, Gemini data loggers, Chichester, UK) buried 10 cm into the soil. Accumulated thermal time above 10°C (a temperature which has been reported at which egg hatch activity ceases[19]) was measured as 278°C days in 2016 and 223°C days in 2017 from sowing to destruction of the brassicas. All plots received 40kg ha^{-1} of nitrogen as ammonium nitrate (NH_4NO_3) in liquid form prior to emergence of the seedlings using a Berthoud raptor FC crop sprayer (Berthoud Agricole SAS, Belleville, France)

Canopy spectral reflectance data were recorded during the growth of the brassicas using a Crop Circle ACS-430 NDVI meter (Holland Scientific, Lincoln, Nebraska, USA) to measure Normalised Difference Vegetation Index (NDVI).[20] We used NDVI as a measure of growth of the trap crops as a higher NDVI equates to greater canopy ground cover and therefore represents greater crop growth, especially as this relationship has been demonstrated in brassica species previously.[21] The brassicas were destroyed early in the following January in both years using a 3 m tractor mounted flail (Maschio Gaspardo S.p.A., Campodarsego, Italy). Prior to flailing a 50x50 cm quadrat was used to take four samples from each plot to determine shoot biomass. Samples were dried at 70°C until constant weight and weighed. After flailing, each plot was resampled for BCN cysts as previously described to determine the *Pf* of each plot and the *Rf* was then calculated as follows:

$$Reproductive\ factor\ (Rf) = \frac{Initial\ population\ (Pi)}{Final\ population\ (Pf)}$$

The length of the experiments (approximately 16 weeks) followed standard farm practice in the UK regarding the cultivation of a cover crop and was to allow for any frost susceptible varieties of the cover crops to die off during the winter rather than result in large amounts of biomass which would have remained on the surface or require the use of herbicides to kill the trap crops. Likewise, the fallow treatments allowed weeds to grow to simulate normal fallow conditions on the fields.

2.4 Data analysis

Data were analysed using Genstat 17th edition (VSN International, Hemel Hempstead, UK). The NDVI data were analysed using a repeated measures ANOVA, and field data analysed using one way ANOVA. Calculation of the least significant difference (LSD) at 5% significance was included in the ANOVA analysis. Figures were prepared using Microsoft Excel (Microsoft Corp, Redmond, Washington, USA) and Graphpad prism version 7 (GraphPad Software Inc. La Jolla, CA, USA)

3. Results

In both years of the field experiments similar trends were observed in the growth of the trap crops as measured using NDVI (Fig1). NDVI increased between the first and second measurements, as the radishes or mustard plants were rapidly growing, and then declined by the third measurement, in early January. Only the fallow treatment shows a continual increase in NDVI values, due to the development of weeds on the fallow plots. In 2016 there were no significant differences in NDVI between trap crops but they had a greater NDVI at 58 DAS than the fallow treatment. In 2017, the susceptible radish had a significantly lower NDVI than the other trap crops at every measurement and resulted in an NDVI at 128 DAS similar to the fallow treatment.

In 2016, the mustard had a significantly greater biomass than all of the other trap crops (Fig 2, $P<0.001$). In 2017 the mustard again shows the greatest development of biomass and was significantly greater than the class 1 radish and class 2 radish B. Class 2 radish A also shows significantly greater growth than class 2 radish B. However, all resistant treatments resulted in greater growth than the susceptible radish in 2017 ($P<0.001$).

When analysing the two years separately, populations of BCN were not significantly reduced by the class one radish or class two mustard compared with the fallow control (2016 $P=0.085$, 2017 $P=0.125$). Equally, the susceptible radish and the two class 2 radish varieties did not cause any clear reductions in BCN populations. However, when data from the two years were combined into a multi-year analysis, significant differences were found between the treatments ($P=0.01$) (Fig 3). The class one radish and the class two mustard showed significant mean population reductions compared to the fallow treatment but differences were not found from using the two class two radish varieties or the susceptible radish.

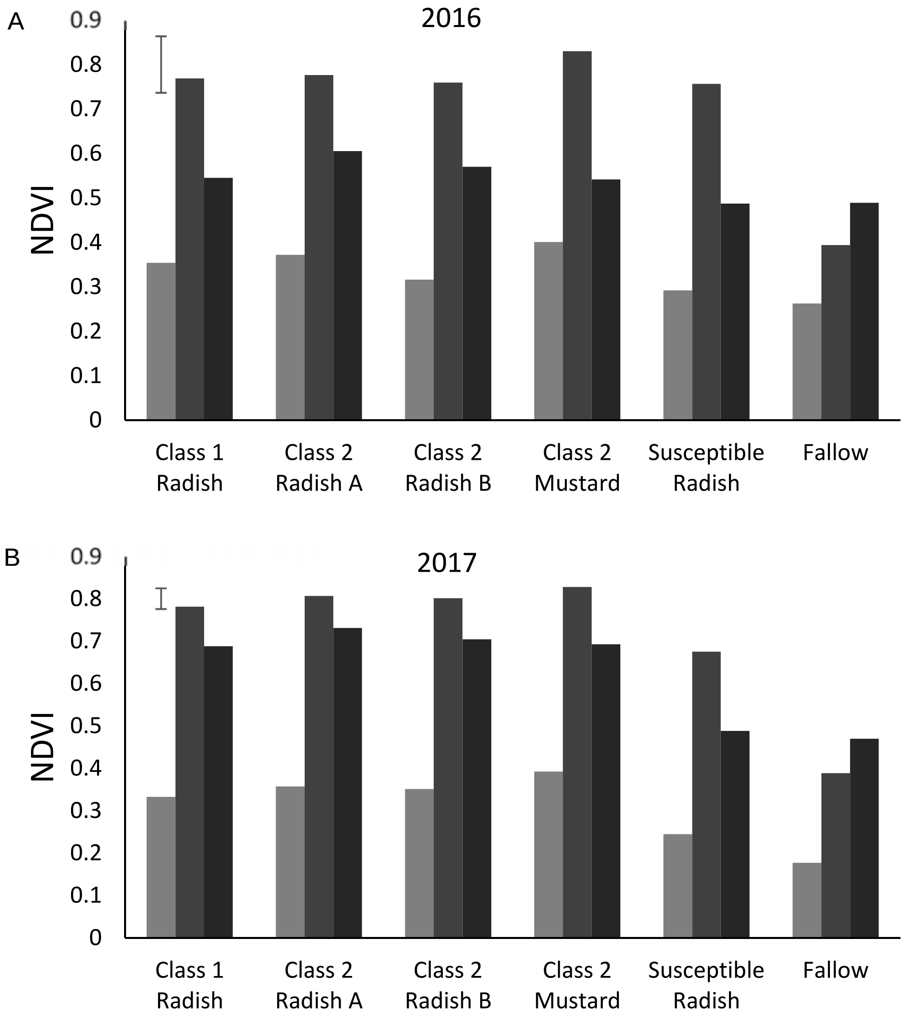

Fig 1. NDVI (Normalised Difference Vegetative Index) of brassica crops grown in fields infested with *H. schachtii* in Norfolk. NDVI was measured three times in each season (2016/17 ■ = 20 DAS [days after sowing], ■ = 58 DAS, ■ = 126 DAS & 2017/18 ■ =23 DAS, ■ = 56 DAS & ■ = 128 DAS) Differences between treatments were found in both years (P<0.001 for both years). Error Bars shows LSD at 5% time x treatment interaction. Fallow treatments were not sterile so NDVI values for these plots shows development of weed plants.

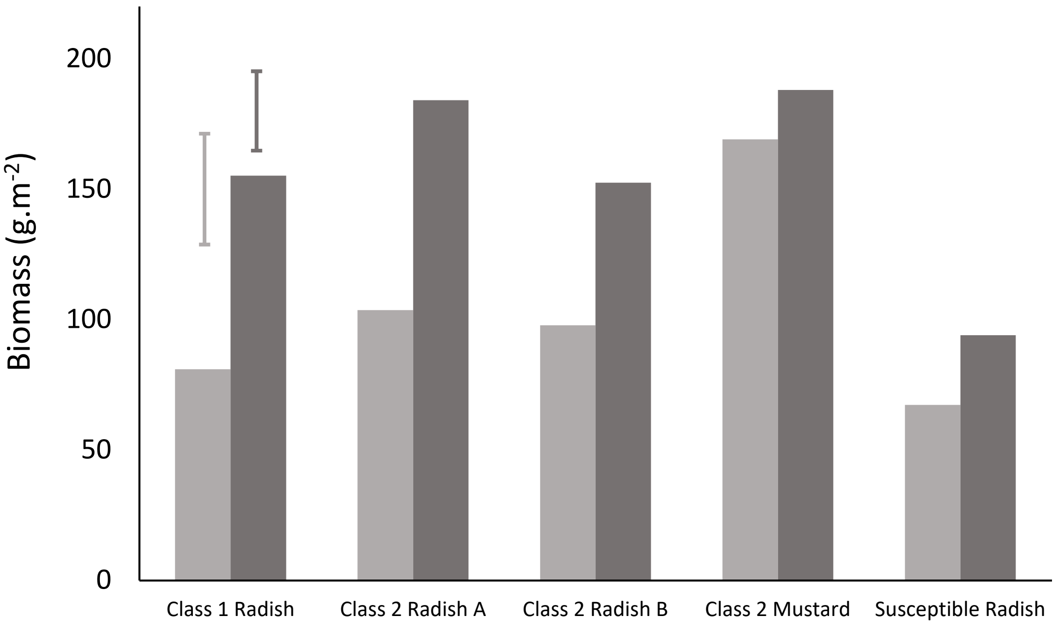

Fig 2. Above ground biomass of the brassica trap crops in 2016 ▒ and 2017 ■ in the field experiments in Norfolk. Significant differences were found in both years ($P<0.001$ in both years) Error bars show LSD at 5% for each respective year. N.B. no samples were taken from the fallow treatments.

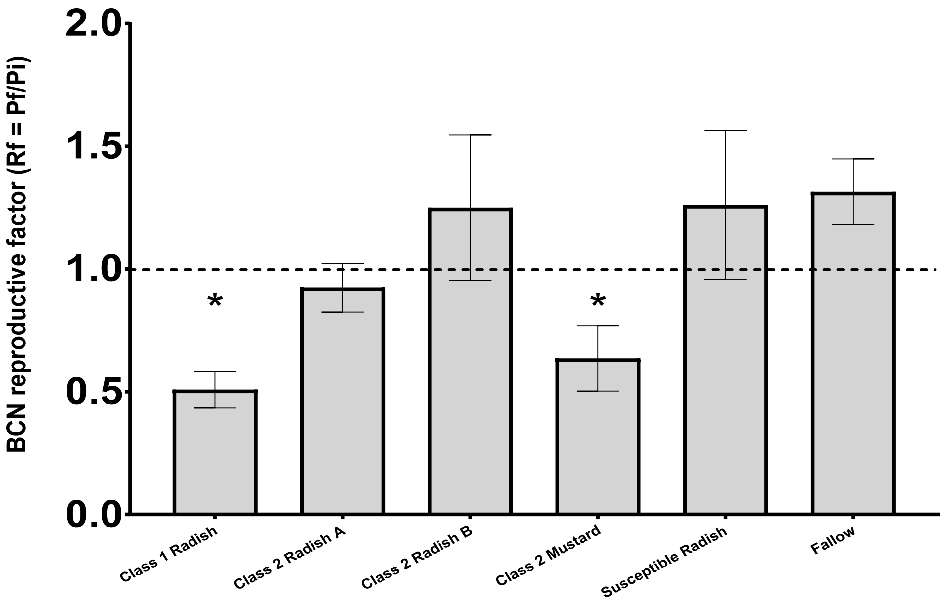

Fig 3. Combined *H. schachtii* reproduction factor data from the brassica trap crops grown in field trials in 2016 and 2017. Significant differences were found between the treatments ($P=0.01$) and significantly reduced populations from the fallow control treatment are denoted by *. The dashed line shows where $Pf/Pi = 1$, i.e. where no net BCN population change was measured. LSD at 5% significance = 0.53. Error bars show ±SEM.

4. Discussion

The interest in the use of resistant brassicas for control of BCN has increased in recent years but limited data has been available on their effectiveness in UK field conditions. We have investigated their use over two field seasons to understand how effective they are at reducing nematode populations and which types are most suited to climatic conditions in Britain.

The class 1 radish and class 2 mustard were the only two treatments to significantly reduce the population of the nematodes when compared to the fallow control and we hypothesise that these varieties stimulate significantly greater levels of BCN hatch when compared to the other varieties. To have found 30 to 40% population reductions from these two varieties when grown for such a short amount of time, and when temperatures have begun to decline, will be important to those dealing with BCN infestations, especially in the UK. Growing class 1 radish and class 2 mustard ahead of sugar beet should reduce the population of BCN, which could infect seedlings and limit growth.

The differences in *Rf* caused by the brassicas are likely explained by a combination of their growth habit and differences in their ability to stimulate BCN juvenile hatch. We found that all of the brassica treatments achieved good levels of ground cover when measured by NDVI, and when the biomass was measured the resistant radishes all seemed to grow equally well in both years, and the mustard also grew well. Therefore, none of the treatments were limited in their growth. As root growth is highly related to shoot growth[22] we can expect that rooting was similar between the treatments and therefore differences likely exist in how stimulating the different varieties are in stimulating BCN J2 hatch. Hatching stimulation must be considered when choosing which variety of trap crop to grow, as this trait might be more important than the resistance rating. Current assessments for resistance to BCN using a bioassay and hatched juveniles might produce misleading results as the ability to stimulate hatch is not assessed. Alternative methods may be needed, such as the use of laboratory hatching assays[23,24] to screen future varieties for their hatching ability combined with confirming resistance to advise farmers with infested fields on which varieties to choose for maximum population control.

The BCN population data show an increase in populations even under the fallow treatment. This was not expected, as we expected populations to remain at levels very close to the *Pi* or to slightly reduce following the fallow treatment, which has been seen in other investigations[14], although Hauer et al.[6] did also find some plots to show large increases in BCN populations under fallow conditions treated with straw mulch. Our increases under fallow could be due to a number of factors. Firstly, BCN susceptible weed species may have been growing and allowed for the population to build up. Weed growth was observed and is reflected in the NDVI measurements on the fallow plots. Fat hen (*Chenopodium album*) is commonly found across the host farm, which is a known host of BCN.[25,26] However, fat hen is classed as a relatively poor host of BCN[27] and Meinecke et al.[25] concluded that common arable weeds do not require control for nematological reasons. Therefore, it seems unlikely that weeds are responsible for our observed population increases although we cannot be certain. Investigations into UK weed populations and their ability to host BCN would be useful to expand on our findings and possibly confirm if the increased populations are due to weeds. A clean fallow treatment was not used in our experiments for two reasons, firstly, we wanted to investigate how a fallow field may cause changes to BCN populations, and secondly, it would not have been viable for the host farmer to regularly spray or cultivate the fallow plots. However, should similar experiments be conducted in the future it would be a good additional treatment and could provide further explanations to our observations.

We hypothesise that consolidation of the soil between the two sampling dates, might have caused the population increases observed. The samples for *Pi* were taken immediately after cultivation of the field in the summer. This created an unconsolidated surface to the soil throughout the area sampled. When the *Pf* samples were taken four months later, considerable weathering due to rainfall had occurred to the soil which led to observed consolidation, i.e. the same mass of soil now filled a smaller volume and lead to soil layers below the 150 mm sample depth in the summer (and therefore not sampled for *Pi*) being included in the winter samples collected to determine *Pf*. This then might have skewed our population counts, especially as BCN populations at deeper layers may be greater than at shallower soil layers[28]. If the findings of other investigations, showing no population build up occurs under fallow[14] and our theory of soil consolidation is correct, it may be the case that both class two radish treatments, and the susceptible radish, did also result in population declines, just that they were not significant enough to detect. However, the results could also be due to sampling error, which is inherently large in field experiments with nematodes[29,30] although this error should have been equal between the plots as the same method was used throughout.

The need to survey the field for BCN populations and then sample each plot before planting the trap crops was very time consuming and also delayed the establishment of the trap crops by several weeks in which the sampling, cyst extraction and nematode counts took place. The effectiveness of the trap crops may be further improved by earlier establishment which would benefit from warmer weather conditions and longer day lengths, thereby promoting more growth of the trap crops and because of these factors earlier sowing has been shown to increase the effectiveness of trap crops and the growth of cover crops in Germany. [6,13,31] However, labour and equipment availability in July and August, typically when harvest of combinable crops is underway, may not allow for earlier sowing. The extension of growing the cover crops through the winter is unlikely to have affected BCN populations due to the temperature of the soil dropping below 10°C, the temperature at which hatching of BCN juveniles ceases[8], during early November in both years.

Population reductions, as a results of growing trap crops may only be useful if they lead to yield increases in following crops and can be accommodated in the farming rotation. Cooke[32] stated clearly that a trap crop should meet three criteria before being suitable for commercial use, and these still stand true today. Firstly it should stimulate egg hatch, which we have shown to be the case with at least two of the varieties used. Secondly, reproduction of the nematode should be limited, which appeared to be the case as no varieties consistently showed major increases in populations. Finally, the trap crop should be agriculturally and economically acceptable. In our case, planting at the beginning of September fitted the farming system on the host farm as cereal harvest was completed and labour and machinery was available for drilling the brassicas. However, when the costs of seed are factored in, along with fertiliser application, and the destruction of the trap crops in the winter, the costs to the grower may be prohibitive and would require the trap crops to boost yields of the following crop of sugar beet by at least 5 tonnes ha^{-1}[i].

The use of BCN tolerant varieties of sugar beet is now common place for growers with infestations.[6,33] These varieties overcome the majority of yield losses, so returns to the grower are significantly greater than using a susceptible variety, but losses are not entirely prevented and a negative relationship between *Pi* and sugar yield has been found by Hauer et al.[6] Therefore, the addition of a trap crop to the rotation may be useful in these circumstances, but only if the additional yield benefit can meet the costs to cultivate the trap crop. Other factors relating to the planting of a trap crop must also be considered and these may be positive (such as improvements to soil structure, weed control, nutrient retention and prevention of soil erosion) or negative, such as providing a habitat overwinter for other agriculturally important pests. Further research is required to consider the use of trap crops within arable farming systems.

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[i] Class 1 radish costs £2.40 per Kg (P. Brown Pers. Comm.) at a seed rate of 22kg.ha^{-1} = £52.80 ha^{-1}. Costs of sowing = 24.37 ha^{-1}, Fertilising 11.16 ha^{-1} (plus fertiliser £21.33) and flailing £19.50 ha^{-1} = Total costs of 129.16 ha^{-1}. With current beet price of £22.50 per tonne, yields need to increase by more than 5.74 tonnes.ha^{-1} following the trap crop to be economically viable. Costs are even higher with other treatments due to greater seed costs. [Costs calculated using standard figures[34]]

5. Conclusions

Our experiments have shown that brassica trap crops may form part of an integrated pest management strategy to manage BCN populations in Eastern England. The class 1 radish and the class 2 mustard treatments tested produced significant population reductions whereas the two class 2 radish treatments did not reduce the populations significantly. Understanding the costs of growing a trap crop and how these might be repaid in the following crop is needed to provide an agronomic benefit.

Further studies are needed to evaluate more of these resistant brassica trap crops over a wider range of seasons and soil types but our results indicate that two varieties tested offer some form of BCN population management. However, these population reductions may soon be undone when a susceptible host species for BCN is planted. In addition, a yield benefit from the use of the trap crop needs to be identified prior to their widespread adoption in Britain for BCN control.

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