# A new treatment for spinach seed with efficacy against seed- and soil-borne fungal pathogens, in particular *Verticillium dahliae*

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## Summary

A commercial development programme has been conducted to generate a new treatment for spinach seed. The focus has been to address the occurrence of *Verticillium dahliae* on spinach seed as well as other seed-borne fungal pathogens. The treatment, GoSeed™, is suitable for organic use (USA-approved). Diagnostic test data are presented for a range of seedlots. The data show that levels of fungal infection differ widely between seedlots, and that several pathogens may be present on individual seeds. The efficacy of a new treatment is evaluated in terms of reduction of on-seed *V. dahliae*. The new process has been designed to be effective against even the highest levels of *V. dahliae* infection. Excellent efficacy against other pathogens such as *Cladosporium variabile* and *Fusarium oxysporum* is also demonstrated. The treatment is also effective against soil-borne pathogens such as *Pythium ultimum* as well as pathogens that are both seed and soil-borne, such as *F. oxysporum*. Seed safety has been a primary consideration. Data are presented showing that treated seed retains germination and, moreover, shows an enhanced speed of emergence.

## Introduction

A programme to generate a commercial spinach seed treatment has been conducted. In order to develop the new treatment, the following aims were established:

- effective treatment of seed-borne *Verticillium dahliae*
- no adverse effect on germination
- acceptability to the organic industry (initially USA)
- efficacy against other seed-borne fungal pathogens
- protection against soil-borne pathogens; seed-borne pathogens are often soil-borne too.

## Methods

Twenty-five different spinach seedlots, mostly different varieties, were solicited under confidentiality from seed producers around the world for testing purposes.

In order to ascertain seed safety, germination testing was conducted according to ISTA methods: 100 seeds per pleat, three replicate pleats, each imbibed with de-ionised water and incubated at 20°C for 14 days. Observations of normal and abnormal emergence were recorded at 42 h and after 14 days. The 42-h count provided an indication of speed of emergence.

To determine on-seed efficacy of the treatment, diagnostics were conducted following the modified freeze-blotter method of du Toit *et al.* (2005). Seeds were imbibed in the dark for

24 h on 10 × 10 cm blotters, 20 seeds per blotter, in sealed square petri dishes, 400 seeds per test. After imbibition, seeds were frozen for 24 h (dark), following which the seeds were incubated at 20°C under a light bank (12/12 h with UV) for up to 14 days. Seeds were examined microscopically for appearance of fungi.

Additionally, pot tests were conducted to challenge seed growth by using artificially inoculated growing media. Planting medium (John Innes seed formulation) was used. *Pythium ultimum* (a causal agent of damping-off) was grown for 7 days on corn meal agar in 9-cm petri dishes and cut into ≈5-mm cubes. Three seeds were sown into each 5-cm pot and a layer of agar cubes with *P. ultimum* was added with the seed prior to covering over with planting medium.

## Results and discussion

A candidate treatment was identified, and treated seed was subjected to germination and diagnostic testing as well as to pot tests. The three samples shown in Figure 1 show little change in the proportion of normal germination as a consequence of the treatment. Similarly, there was little change in the proportion of abnormal germination. However, early emergence

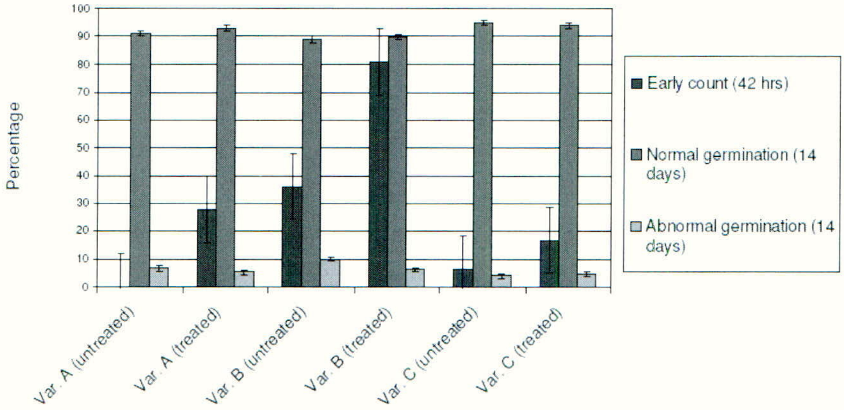

**Figure 1** Germination of three varieties before and after treatment (±SE)

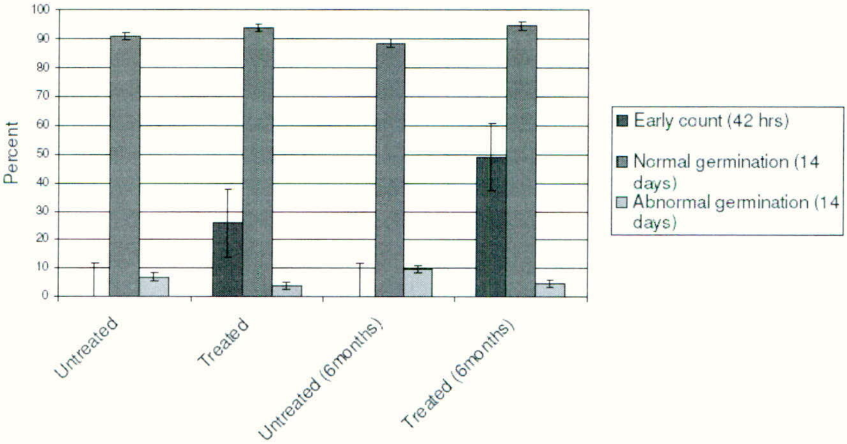

**Figure 2** Variety A: 6-month storage of treated and untreated seed (±SE)

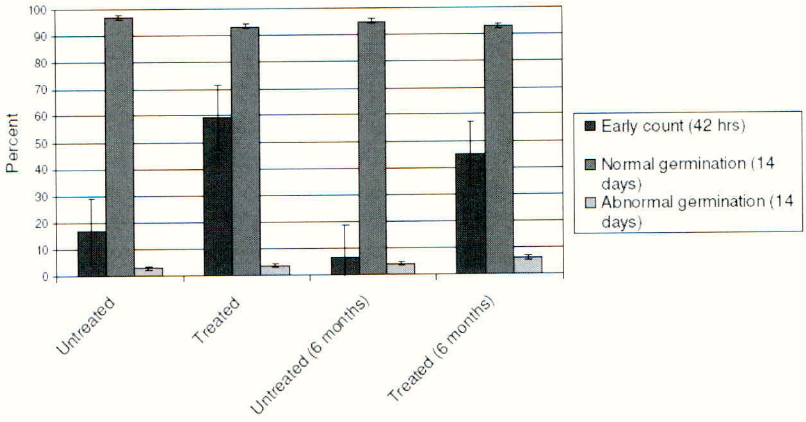

**Figure 3** Variety B: 6-month storage – treated *vs* untreated (±SE)

was improved in all varieties, significantly so for two of the three varieties shown. Variety A, as an example of real-time storage for 6 months at 15°C (Figure 2), shows that for treated seeds there was a slight increase in the proportion of normally germinating seed. There was a slight reduction in abnormally germinating seed. The improvement in early emergence seen in treated seed was retained even after 6 months' storage, and actually showed an increase to result in a proportion approaching 50%. Variety B, as a second example of real-time storage, again for 6 months at 15°C (Figure 3), showed little change in the proportion of normally germinating seeds after 6 months. There was a slight reduction in the proportion of abnormally germinating seed. Early emergence had increased after 6 months' storage to give a figure of just over 45%. The two examples show a good indication that the treated seed has stored well for 6 months.

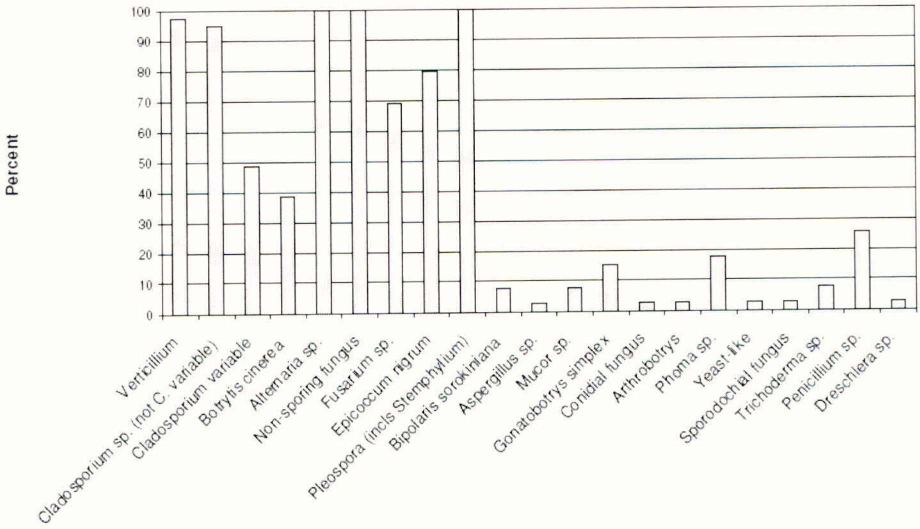

**Figure 4** Frequency of fungi across all untreated seedlots

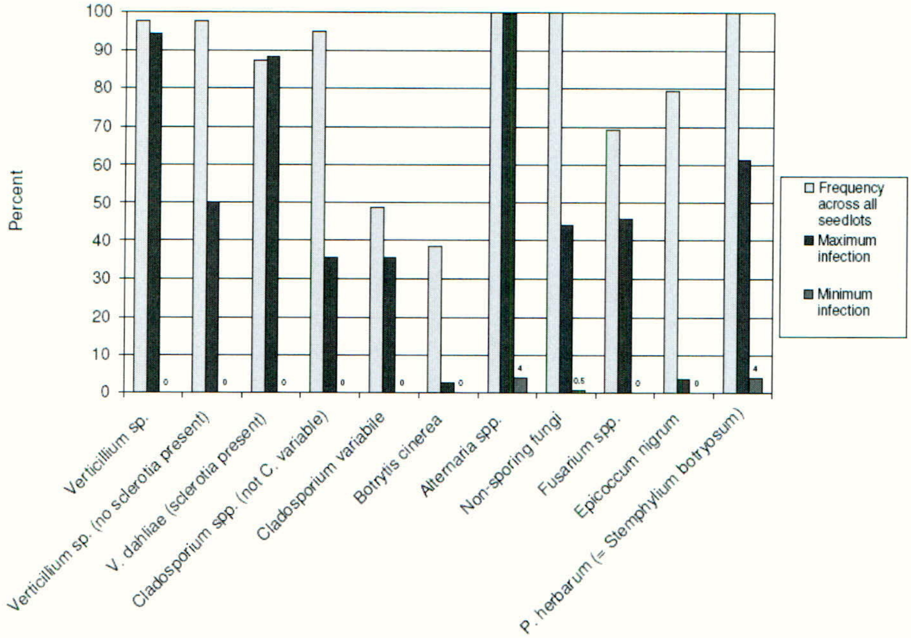

**Figure 5** Fungi on untreated seed: maximum and minimum levels of infection within seedlots

In order to determine the efficacy of the treatment, diagnostics were conducted on all samples. In total over 60,000 seeds were examined, for which data on all fungi observed were individually scored.

Figure 4 shows the frequency of fungi across the 25 untreated seedlots. The fungi on the left are those that were most frequent among the seedlots; the right-hand side shows those of lesser frequencies. The listing is not exhaustive, but serves to illustrate that untreated seed is populated by a wide range of fungi, with a wide range of ecological roles ranging from pathogens to beneficials.

When considering the maxima and minima, on any given seedlot, of the fungi most frequently present across all seedlots (Figure 5), it can be seen that the ranges varied considerably. For example, on some seedlots *Verticillium* was present at levels approaching 100% of seed infected, but in other seedlots was completely absent. The only taxa present on all seedlots were *Alternaria* spp. and *Pleospora herbarum*.

As an example of the treatment efficacy, variety A (Figure 6A) showed initially low levels of most fungi, with the exception of *Alternaria* spp. and *Stemphylium botryosum*. After treatment, levels of all fungi were reduced. *Verticillium dahliae*, the main target, was present only at about 15% initially, but this was reduced to 2.5% post-treatment. *Stemphylium botryosum*, a secondary target for the treatment, was reduced by 58% to about 18%. A second example, variety B (Figure 6B), showed that most of the fungi present initially were at comparatively low levels, with the notable exception of a very high level for *V. dahliae*. After treatment, most fungi were reduced to zero, including *V. dahliae*. A third example, variety C (Figure 6C) initially had high levels of *Alternaria*, *Stemphylium* and *Fusarium* spp. *Verticillium dahliae* was also present, but at a lower level than in variety B. After treatment, *Alternaria* spp. and *S. botryosum* were reduced to very low levels, whilst *V. dahliae* was reduced to zero.

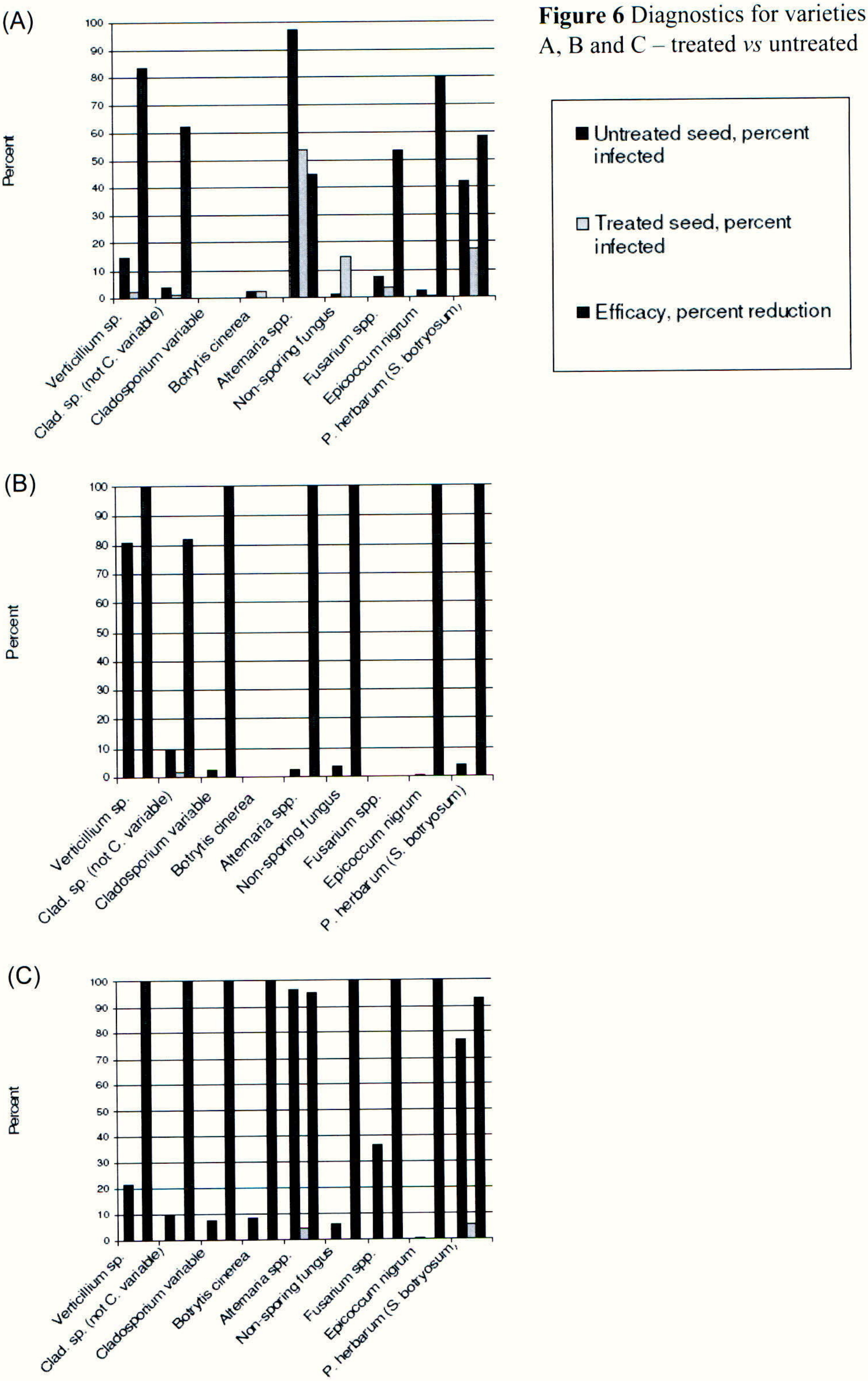

**Figure 6** Diagnostics for varieties A, B and C – treated *vs* untreated

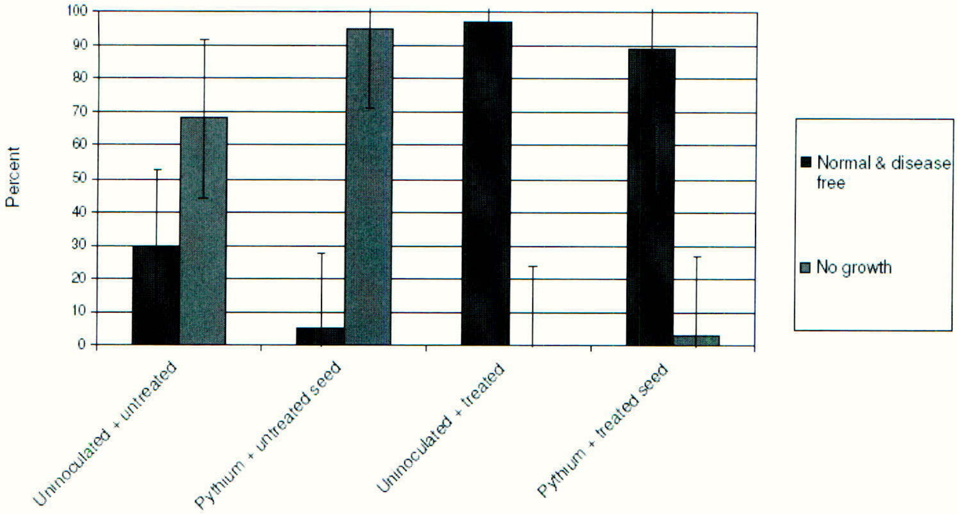

**Figure 7** Pot experiment – inoculated with *Pythium ultimum*, seedlings at 14 days

The diagnostic tests have shown that the species present on untreated seed can vary, and the levels of infection of those particular fungi can also vary. Nonetheless, our treatment has been very effective against the main target, *V. dahliae*, as well as *Cladosporium variabile* and *Fusarium* spp., and it has also shown good efficacy against *S. botryosum*.

Figure 7 summarises data from one of the pot tests. For the uninoculated pots, 30% of untreated seed showed normal emergence after 14 days, whereas ≈97% of the treated seed showed normal emergence in uninoculated pots. With *P. ultimum* present, only 5% of the untreated seed showed normal emergence, whilst treated seed showed ≈89% normal emergence.

## References

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# Pest and virus control in winter oilseed rape in northern Europe using a clothianidin-based seed treatment

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## Summary

To meet the challenges of more severe and diverse pest pressures in establishing oilseed rape crops, a new clothianidin + beta-cyfluthrin seed treatment, applied at 5g + 1g a.i. per kg seed, has been developed for use in northern Europe. Results demonstrate that the clothianidin-based treatment gives enhanced crop establishment and leaf protection against damage caused by adult cabbage stem flea beetle. In addition, the seed treatment gives useful reduction in damage caused by the larvae of turnip sawfly and cabbage root fly. Very good control of peach potato aphid is achieved, resulting in substantial reductions in the levels of turnip yellows virus in crops that were not subsequently re-infected by early spring aphid migrations.

## Introduction

Oilseed rape is a major arable crop in northern Europe. Its importance is not just as a cash crop, the value of which has increased significantly over the past few years along with other commodity prices, but also as the principal break crop in what would otherwise be cereal monocultures. This demand has been driven by an increase in the consumption of rapeseed oil as a source of biodiesel in the EU and the rest of the developed world. The yields of oilseed rape crops, however, have hardly increased at all during the past 20 years, despite genetic advances, and the causes of the suppression of yield potential have been attributed to fungal diseases (Booth, 2008) and viral diseases (Stevens *et al.*, 2008).

It has long been recognised that adult cabbage stem flea beetle (*Psylliodes chrysocephala*) could, under certain circumstances, be a major pest of emerging and establishing winter oilseed rape crops. Following the banning of gamma HCH, the first neonicotinoid-based seed treatment, Chinook® (imidacloprid + beta-cyfluthrin) was introduced in Europe in the early 2000s. This treatment gave effective short-term protection against adult cabbage stem flea beetle damage, and remains widely used. However, the increasing frequency of long, dry and relatively mild autumns in northern Europe has resulted in increased economic damage being caused by a more diverse range of hitherto ‘minor’ pests. A new seed treatment suitable for these more challenging conditions has been developed, based on the neonicotinoid insecticide clothianidin and the pyrethroid beta-cyfluthrin, and this was recently registered in the UK and other EU countries under the trade name Modesto®. This paper summaries some of the more than 100 field trials conducted in Europe in the development of this seed treatment from 2003 up until the current season.

## Materials and methods

The clothianidin and beta-cyfluthrin seed treatment (CTD&CYB), applied at a rate of 5 g+1 g a.i. per kg seed was tested in comparison with untreated seed and seed treated with imidacloprid with beta-cyfluthrin (IMD&CYB), applied at the commercial rate of 2 g+2 g a.i. per kg seed. Small-scale applications were applied using laboratory-scale spinning disc batch applicators such as the 'Mini-Rotostat' or 'Norogard'. Commercial batch applicators were used for the larger field trials. Immediately after the introduction of the liquid seed treatment, talcum powder or a similar material was applied to dry and separate the treated seed, and to allow it to flow freely through applicators and seed drills.

### *Efficacy and crop safety trials*

Over 100 trials were carried out throughout northern Europe (UK, France, Germany, Sweden and Poland) between 2003 and 2009, targeting a range of pests and conditions. Trial plots were drilled using Hege- or Wintersteiger-type plot drills, with plot sizes ranging from 10 to 40 $m^2$ and normally incorporating four replicates per treatment (range three to six replicates). Where pests were active, the efficacy of the treatments was determined by assessing one or more of: the plant stand; the numbers of larvae or adult individuals per species present; the percentage leaf area or numbers of leaves or roots damaged. Root damage was also assessed for incidence and severity using a 0–5 score where 0 = 0%; 1 = 1–10%; 2 = 11–30%; 3 = 31–50%; 4 = 51–75%; 5 = >75%. The presence of *Turnip yellows virus* (TuYV), formerly known as *Beet western yellows virus* (BWYV), was determined by assessing 20 leaves per plot using ELISA tests carried out at IACR Brooms Barn in Suffolk, UK. Trials were harvested using standard plot combines and grain moisture corrected to 89% DM.

## Results

Crop safety results are covered by Adam & Hopkinson (2008). Crop establishment counts from trials where insect pest damage was observed are given in Table 1. Reductions in crop stand were attributed to damage caused by adult cabbage stem flea beetle in the majority of trials. At two sites the causal pest was the larva of turnip sawfly (*Athalia rosea*).

Direct observations of damage caused by cabbage stem flea beetle (from UK, France, Germany and Sweden) are given in Table 2, and by turnip sawfly (from UK, France and Germany) in Table 3.

**Table 1** Mean number of established oilseed plants in insect-infested crops treated with insecticide seed treatments compared with untreated control (25 results)

| | **Plants/m row (assessed at 1–3 true leaf stage)** | | |
|---|---|---|---|
| **Treatment a.i./kg** | **Mean** | **Range** | **Relative** |
| Untreated | 9.3 | 3.3–25.9 | 100.0 |
| IMD&CYB 2 g+2 g | 10.2 | 3.7–25.1 | 109.7 |
| CTD&CYB 5 g+1 g | 10.6 | 3.1–24.3 | 114.0 |

A pest of particular importance in northern Germany is cabbage root fly (*Delia radicum*). As the larvae were active in October and November of each year, the assumption is made that they were the product of third-generation cabbage root fly. Trial results (primarily from Germany) are given in Table 4.

**Table 2** Leaf damage caused by adult cabbage stem flea beetle (*Psylliodes chrysocephala*) assessed primarily at 1–3 true leaf stages (range 1–5 true leaves): percentage plants damaged (20 results); numbers of feeding holes per plant (11 results)

| | **Percentage plants damaged** | | **Feeding holes per plant** | |
|---|---|---|---|---|
| **Treatment a.i./kg** | **Mean** | **% reduction** | **Mean** | **% reduction** |
| Untreated | 38.0 | 0.0 | 3.9 | 0.0 |
| IMD&CYB 2 g+2 g | 23.3 | 38.7 | 2.1 | 46.2 |
| CTD&CYB 5 g+1 g | 16.2 | 57.4 | 1.0 | 74.4 |

**Table 3** Leaf damage caused by larvae of turnip sawfly (*Athalia rosea*) assessed primarily at 3–4 true leaf stages (range 1–7 true leaves): percentage leaf area damaged (7 results); % larval infestation (7 results)

| | **Percentage leaf area damage** | | **Percentage larval infestation** | |
|---|---|---|---|---|
| **Treatment a.i./kg** | **Mean** | **% reduction** | **Mean** | **% reduction** |
| Untreated | 22.0 | 0.0 | 65.6 | 0.0 |
| IMD&CYB 2 g+2 g | 15.2 | 30.9 | 34.3 | 47.7 |
| CTD&CYB 5 g+1 g | 9.8 | 55.4 | 10.7 | 83.7 |

**Table 4** Root damage caused by cabbage root fly larva *Delia radicum* (Germany, Poland) assessed primarily at 7–9 true leaf stages (range 6–10 true leaves): percentage plants damaged (9 results); root damage index (7 results)

| | **Percentage plants damaged** | | **Root damage index** | |
|---|---|---|---|---|
| **Treatment a.i./kg** | **Mean** | **% reduction** | **Mean** | **% reduction** |
| Untreated | 39.7 | 0.0 | 1.3 | 0.0 |
| IMD&CYB 2g+2g | 30.4 | 23.4 | 0.9 | 30.8 |
| CTD&CYB 5g+1g | 19.7 | 50.4 | 0.6 | 53.8 |

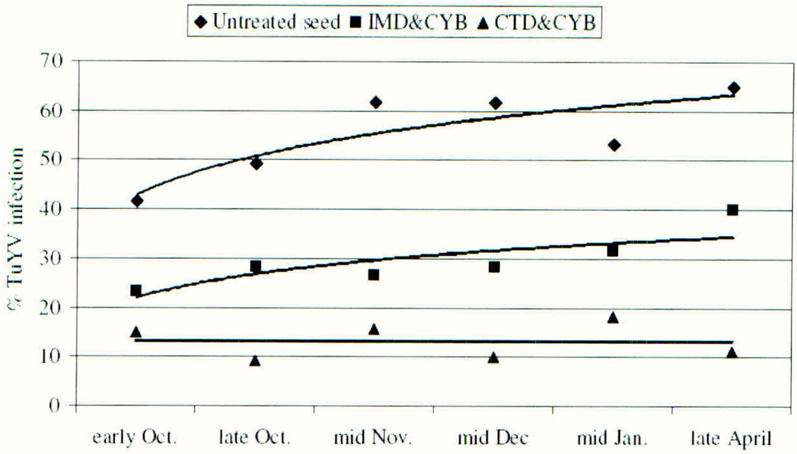

**Figure 1** Seasonal development of *Turnip yellows virus* (TuYV) in oilseed rape: mean of three trials in England, 2007–08

The peach potato aphid (*M. persicae*) rarely builds up sufficient numbers in winter oilseed rape crops to cause physical damage, however it is the principal vector of TuYV. The aphid counts from UK and French trials are given in Table 5. The TuYV ELISA test results from trials in England are given in Table 6, and are split between results with infection levels above or below 85%. More detailed studies were undertaken in 2007–08 when the seasonal

**Table 5** Reduction in: potato peach aphid infestation *Myzus persicae* (from UK, France) (10 results) assessed primarily at 5–7 true leaf stages (range 3–9 true leaves)

| | **Aphids per plant** | | |
|---|---|---|---|
| **Treatment a.i./kg** | **Mean** | **Range** | **% reduction** |
| Untreated | 2.81 | 0.22–6.40 | 0.0 |
| IMD&CYB 2 g+2 g | 0.67 | 0.00–2.06 | 76.2 |
| CTD&CYB 5 g+1 g | 0.15 | 0.00–0.44 | 94.7 |

**Table 6** Percentage plants infected with *Turnip yellows virus* (TuYV) where virus <85% (7 results); percentage plants infected with TuYV where virus >85% (8 results); UK trials assessed in the spring following sowing at early stem extension stage

| | **TuYV < 85%** | | **TuYV > 85%** | |
|---|---|---|---|---|
| **Treatment a.i./kg** | **Mean** | **% reduction** | **Mean** | **% reduction** |
| Untreated | 44.0 | 0.0 | 96.6 | 0.0 |
| IMD&CYB 2 g+2 g | 19.8 | 55.0 | 83.8 | 13.3 |
| CTD&CYB 5 g+1 g | 10.6 | 75.9 | 71.1 | 26.4 |

**Table 7** Mean crop yield from five trials where *Turnip yellows virus* (TuYV) had been confirmed, from UK (2005–08 harvest seasons)

| | **Crop yield (t/ha)** | | |
|---|---|---|---|
| **Treatment a.i./kg** | **Mean** | **Range** | **Relative** |
| Untreated | 3.23 | 2.58–3.76 | 100.0 |
| IMD&CYB 2 g+2 g | 3.48 | 2.51–4.2 | 107.8 |
| CTD&CYB 5 g+1 g | 3.61 | 2.90–4.0 | 111.8 |
| % TuYV | 66.5 | 21.3–100 | |

development of turnip yellows virus was recorded in three trials in England at approximately monthly intervals from 3 weeks after crop emergence. The mean results are depicted in Figure 1, to which calculated trend lines have been added.

## Discussion

Given the financial and agronomic importance of the oilseed rape crop in the UK and the rest of northern Europe, it is critical that the crop achieves good establishment so that it has the potential to return a good yield. Preventing or at least reducing damage from adult stages of cabbage stem flea beetle is crucial to obtaining good crop establishment. Insecticide seed treatments are the most effective method of affording that protection, as this pest can cause severe damage to the crop even before it emerges from the ground. The results given in this paper demonstrate that the clothianidin-based seed treatment improves crop establishment in the presence of soil-dwelling pests, particularly cabbage stem flea beetle, over that given by the imidacloprid-based treatment. Not only that, but the direct assessments of cabbage stem flea beetle damage, which were generally conducted some time after crop emergence counts, revealed that the clothianidin-based treatment gave greater persistence in protecting the crop.

Changes in the climate have, in the main, been notable for the warmer and drier summers which have extended into milder and longer autumns, followed by winters with fewer frosts. Conditions such as these have favoured the build-up of aphid populations (Stevens *et al.*, 2008); extended the potential for third-generation cabbage root fly to damage crops (Anon., 2004) as experienced in Germany; and resulted in damage by turnip sawfly larvae reaching economic proportions in parts of England. The clothianidin-based seed treatment gives very useful activity against both turnip sawfly and cabbage root fly which, in many instances, would have reduced the requirement for foliar insecticides to be applied to the affected crops.

The insecticide clothianidin is very effective when applied as a seed treatment in giving protection against the peach potato aphid in arable crops. To date, there have been no recorded instances of resistance in this aphid species against the neonicotinoid insecticides in northern Europe, unlike the situation with regard to the increased levels of resistance against the pyrethroid and carbamate insecticide groups (Foster & Denholm, 2008). The trials results reported demonstrate that the clothianidin-based seed treatment is particularly effective in giving protection against aphid infestation in oilseed rape. This is very important because the peach potato aphid is one of the most important vectors of turnip yellows virus, and research has shown that up to 70% of winged aphids caught in traps are carrying the virus. TuYV infections of up to 100% have been recorded in commercial crops in the UK. The presence of the virus can only be confirmed by ELISA tests, so the infection goes undetected in most commercial crops. Although visually difficult to recognise, the virus has significant effects on infected crops, such as reduction in stem height, leaf area, raceme and pod numbers, and reduction in the number of seeds per pod. Yield losses of 10–40% have been recorded (Stevens *et al.*, 2008).

The results reported in Table 6, where TuYV was less than 85% infection, demonstrated a mean reduction of over 70% from use of the clothianidin-based seed treatment, and this was reflected in yield increases (Table 7). The virus testing was initially conducted in the spring following sowing as the crops entered the stem extension phase, and it was noted that virus control from the clothianidin-based seed treatment was sometimes less effective. In an attempt to understand the dynamics of virus infection, a small number of trials were established in 2007–08, and plants were sampled at monthly intervals over the autumn and winter. Within 3 weeks of crop emergence, 40% of unprotected plants had already been infected with TuYV, and this rose to over 60% by the following January. By late April, the virus levels on unprotected plants had risen only marginally as the aphid migration had all but ceased. This was in contrast to the results reported by Stevens *et al.* (2008) for 2006–07, when the aphid migration continued until April and the TuYV level rose steadily through the early spring. It is concluded therefore that the apparently poor levels of virus control from trials with greater than 85% infection, principally in 2006–07, were due to the early spring aphid migration when the seed treatment had naturally degraded and been diluted in the growing crop.

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