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PROJECT REPORT No. OS52

REDUCING DAMAGE TO OILSEED RAPE BY WOODPIGEONS:
I. TRIALS WITH CINNAMAMIDE;
II. BREEDING HIGH GLUCOSINOLATE VARIETIES

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REDUCING DAMAGE TO OILSEED RAPE BY WOODPIGEONS: I. TRIALS WITH CINNAMAMIDE; II. BREEDING HIGH GLUCOSINOLATE VARIETIES

by

J V COTTERILL[1], D P COWAN[1] AND R MITHEN[2]

[1]Central Science Laboratory, Sand Hutton, York, YO40 1LZ

[2]John Innes Centre, Norwich Research Park, Colney Lane, Norwich, NR5 7UJ
(Now University of Nottingham, Sutton Bonington Campus, College Road, Sutton Bonington, Loughborough, Leicestershire LE12 5RD)

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Abstract

The aim of the project was to evaluate two methods of reducing woodpigeon damage to oilseed rape by reducing the palatability of the crop: through the application of an avian repellent, cinnamamide, and modification of rape leaf glucosinolates.

Cinnamamide:

In a pilot field trial carried out prior to the current project, cinnamamide was shown to reduce pigeon damage to rape, however, the persistence of cinnamamide on the leaves was poor. A more weather-resistant suspension concentrate (SC) formulation of the avian repellent cinnamamide, has been developed. Studies of this improved formulation, which uses a 10 % Acronal / 2 % Emoleo L1 mix in the ratio 3:1 v/v as the sticker, suggested that leaching would be less than half that of the original formulation (the inclusion of Emoleo L1 was also beneficial as it reduces leaching associated with freezing and thawing cycles). A bioassay using captive pigeons showed that the modified stickers do not mask the repellent properties of cinnamamide; hence the improved formulation was suitable for use in subsequent field trials. Trials carried out over three years showed that the persistence of the new cinnamamide formulation has been greatly improved compared to that used in the pilot trial.

In the final field trial there was sufficient woodpigeon damage to the field site to assess the effectiveness of the improved cinnamamide formulation and compare it with the high glucosinolate JIC line of rape. Cinnamamide was shown to reduce the level of pigeon damage to rape, and towards the end of January treated plots had only 73 % of the damage found on control plots. Although the cinnamamide did not keep the pigeons off the plots altogether, there was a significant reduction in the number of plants which were severely damaged. It is these severely damaged plants which are least likely to recover, and so have the greatest effect on yield. The JIC rape was also shown to be less palatable to pigeons. Plots with the "double protection" of the cinnamamide spray and the high glucosinolate rape were the least damaged. By the end of January, plots of the JIC rape which were also sprayed with cinnamamide, had only 70 % the damage of untreated Apex plots.

The project has demonstrated that when cinnamamide is present on leaf surfaces, the amount of severe pigeon damage is reduced. As such it would provide an effective and environmentally benign means of reducing woodpigeon damage to oilseed rape. Although cinnamamide is not yet registered for use as a repellent on oilseed rape, industrial sponsorship is currently being pursued to take it through to the registration procedure.

Glucosinolates:

Winter oilseed rape double haploid lines have been developed which have enhanced levels of 2-propenyl glucosinolates and 3-butenyl glucosinolates in their leaves, but which retain low seed glucosinolates (GLS's). From small plot trials, lines with good GLS profiles, which showed a reduction in pigeon grazing and which yielded within the normal range of advanced breeding lines, were selected for larger scale field trials. In 1999/2000, a number of fields at farms across Britain were sown with either pure modified GLS line (JIC1) or as a mixture with other double low cultivars. At the majority of sites, a reduction in pigeon grazing was observed in these fields compared to surrounding fields, where pigeon damage was significant. It is concluded that the development of OSR with enhanced glucosinolates has the potential to reduce pigeon damage, and that it is most likely to be viable in practice if used as a mixture with the latest high yielding lines.

SUMMARY

Woodpigeons (*Columba palumbus L.*) cause significant damage to oilseed rape in Britain, especially between January and March. One method of reducing the damage caused is to reduce the palatability of the crop to the pigeons. Two different approaches to this have been investigated in the project. Firstly, by application of the avian repellent compound cinnamamide and, secondly, by modification of rape leaf glucosinolates.

Cinnamamide:

In a previous pilot field trial on autumn-sown oilseed rape, a suspension concentrate formulation of the avian repellent cinnamamide, using Acronal 4D (5 % w/v) as the sticker, was shown to reduce damage by woodpigeons. However, the persistence of cinnamamide on the rape leaves was poor. The aim of project was to develop a more weather-resistant formulation of cinnamamide.

The use of a more effective sticker in the suspension concentrate formulation was identified as potentially the most likely means of improving the persistence of cinnamamide on oilseed rape. *In vitro* studies were designed to assess the leaching of cinnamamide (identified as being the major mechanism of loss of the repellent). Leaching of cinnamamide was assessed by washing deposits of formulations applied to glass slides or sprayed onto rape leaves, using a “rainwashing machine”. A number of stickers were evaluated and the effects of the sticker concentration, ageing of the deposits and freezing on “rainfastness” were investigated. These studies indicated that the persistence of cinnamamide could be greatly improved by increasing the concentration of Acronal 4D and by inclusion of a second sticker Emoleo L1, to reduce the negative impact of freezing on rainfastness. These studies indicated that by using a formulation of 10 % Acronal with 2 % Emoleo L1 (ratio of 3:1 v/v), the amount of cinnamamide washed off the leaves would be less than half that of the original formulation.

A bioassay using captive pigeons was carried out to establish appropriate application rates for cinnamamide and to test the repellency of four modified formulations. An optimum application of 3 kg/ha of cinnamamide was identified for future trials. A two-choice bioassay showed that the modified stickers in the formulation did not mask the repellent properties of cinnamamide compared to the original sticker. Hence the sticker identified from the *in vitro* studies as having the best rain-fastness (10 % Acronal with 2 % Emoleo L1 (ratio of 3:1 v/v), was selected for use in field trials.

The improved formulation was used in a field trial in Kent in 1997/1998, and the persistence of cinnamamide was shown to be greatly improved compared to the pilot field trial. Although there was only a low level of pigeon damage to the site, the cinnamamide treated plots did receive less damage than the control plots.

A second field trial was carried out in 1998/1999, to compare the efficacy of the cinnamamide formulation with a modified glucosinolate line of oilseed rape provided by John Innes Centre (JIC 1). Unfortunately there was almost no pigeon damage at all to the plots, so no further assessment of the efficacy of cinnamamide as a feeding deterrent could be made. However, the persistence of cinnamamide using the modified formulation was determined on the standard cultivar and the JIC modified line. This was again shown to be improved substantially compared to the original pilot field trial.

An extension to the project allowed for a further trial to compare the two potentially powerful means of reducing pigeon damage to rape. Replicate blocks of Apex and JIC modified breeding line were drilled, half of which were sprayed with cinnamamide and half left un-sprayed. Fortunately a substantial amount of pigeon damage occurred to the field site, which allowed for a proper assessment of the improved cinnamamide formulation and the JIC modified breeding line. Although there was already a significant amount of pigeon damage to the plots before the first cinnamamide application in December, damage to cinnamamide treated plots was less than on un-sprayed plots. Towards the end of January, the cinnamamide plots had significantly less damage than the control plots. It was also encouraging that there was a significant reduction in the proportion of plants which had severe damage in sprayed plots, with more of the plants receiving no or negligible damage. It is the severe damage to plants, in particular when the inner growth points are removed, from which the plants are least likely to recover which thus has the greatest potential impact on yield. Residue analysis showed that the persistence of cinnamamide on the leaves was again greatly improved compared to the pilot trial.

Comparisons of Apex and the JIC line (JIC1) showed that the high glucosinolate rape was less palatable to pigeons, particularly between December and January. The plots which received the least damage, were however, those with the combined “double protection” of cinnamamide and JIC line of rape. At the end of January, these plots had approximately 70 % the damage of un-sprayed Apex plots. The trial plots were taken on to harvest. However, the reduced pigeon damage to cinnamamide treated plots and JIC plots wasn’t reflected in an increase in yield, there being no significant difference in yield between the plots. This may have been due to a considerable amount of damage to the treated plots occurring before the first cinnamamide spray, the ability of the crop to recover from a certain amount of pigeon damage, and to the many other factors which affect yield other than pigeon damage. However, from the yield assessments from the last two year’s field trials, it is encouraging that there is no apparent yield penalty or phytotoxicity with the cinnamamide spray application. Despite the reduction in pigeon damage to cinnamamide treated plots not being reflected in a higher yield, the major objective of the project, of developing a cinnamamide formulation with improved persistence that significantly reduces damage was achieved. We have shown that when cinnamamide is present on the rape leaves, it is effective at reducing the severity of the damage caused by pigeons. Although it is not yet registered for use as a repellent on brassicas, it would provide an effective and environmentally benign means of reducing woodpigeon damage to oilseed rape.

Glucosinolates:

As generalist pests have been shown to be deterred by increased levels of glucosinolates (GSLs) in the leaves of oilseed rape (Giamoustaris & Mithen 1995), the idea of breeding agronomically acceptable lines of rape with elevated or altered GSL levels to deter these birds is attractive. These lines would offer a low-maintenance insurance mechanism for farmers as they would have built-in defences against the pigeons.

Winter oilseed rape double haploid lines have been developed which have enhanced levels of 2-propenyl glucosinolates and 3-butenyl glucosinolates in their leaves, but which retain low seed glucosinolates (GLS's). A summary of the breeding programme is presented. From small plot trials, lines with good GLS profiles, which showed a reduction in pigeon grazing and which yielded within the normal range of advanced breeding lines, were selected for larger scale field trials.

In 1996/1997 a field trial in Kent showed that the F3210 line had significantly higher total GLS's than the Apex control, and suffered less damage in the worst affected plots. It also had a much higher proportion of 3-butenyl and 2- propenyl GSL's which have previously been implicated as being important in palatability to herbivores. In 1997/1998 and 1998/1999, trials were carried out at four different sites across the country. Unfortunately there was almost no pigeon damage to any of the trial sites in these years, so no further assessment of palatability to pigeons could be made. However, the plots were taken to harvest in the 1998/1999 trials and it was encouraging that one of the lines (JIC1) yielded not significantly less than Apex at any of the sites.

In 1999/2000, a number of fields at farms across Britain were sown with either pure modified GLS line (JIC1) or as a mixture with other double low cultivars. There was a significant level of pigeon damage to the trial sites this year, from which a qualitative assessment of pigeon damage was made to assess palatability of the JIC1 line. At the majority of sites, a reduction in pigeon grazing was observed in these fields compared to surrounding fields, where pigeon damage was significant. The complexity of analysing the pigeons' responses to these lines in field trials and aspects of experimental design are discussed. It is concluded that the development of OSR with enhanced glucosinolates has the potential to reduce pigeon damage, and that it is most likely to be viable in practice if used as a mixture with the latest high yielding lines.

I. TRIALS WITH CINNAMAMIDE

1. INTRODUCTION

Woodpigeons (*Columba palumbus L.*) cause significant damage to brassicas in Britain, in particular to autumn sown oilseed rape (Murton & Jones, 1973; Inglis et al., 1989). Rape is especially vulnerable in its early stages of growth between January and March, when alternative food sources are scarce (Inglis et al., 1990). At present, no single method of control currently exists that reliably and significantly reduces pigeon damage to autumn sown oilseed rape. The project was designed to evaluate two potentially powerful methods of reducing the palatability of oilseed rape to pigeons, by application of the avian repellent cinnamamide and through modification of leaf glucosinolates (see SECTION II)

Extensive previous research at the Central Science Laboratory has shown that cinnamamide, a synthetic derivative of the plant secondary compound cinnamic acid, is an effective feeding deterrent for a variety of bird species (Crocker & Reid, 1993, Crocker et al., 1993, Watkins et al., 1994). In a pilot field trial, cinnamamide was shown to reduce woodpigeon damage to oilseed rape plants (Gill et al., 1998). However, although the results were promising, the effects were of limited duration. This was attributed to the late timing of the application and poor persistence of cinnamamide on the leaves. After application the compound was subjected to heavy precipitation, freezing and high winds. Residue studies showed that levels of cinnamamide on the leaves declined by 71 - 94 % after only 3 days, being undetectable after 13 days. Clearly there was a need for a more weather resistant formulation.

Foliar applied pesticides are susceptible to a number of processes which can reduce residues on leaf surfaces, including volatilisation, photodegradation, plant uptake, mechanical detachment and leaching by rain (Barlow, 1985, Sundaram, 1995). For cinnamamide to be effective in reducing damage to oilseed rape by woodpigeons, it has to persist on the leaves during often harsh winter conditions. The challenge is to develop a formulation that yields persistence under these circumstances without compromising cinnamamide’s avian repellent properties.

The research carried out during the project is presented here in three sections – firstly, the formulation development, secondly, bioassays using captive pigeons to assess the repellency of the improved formulation and, thirdly, field trials carried out to assess the efficacy of the cinnamamide formulation in the field.

2. DEVELOPMENT OF WEATHER-RESISTANT FORMULATION

The formulation used in the pilot trial was a suspension concentrate (SC), consisting of finely ground cinnamamide dispersed in water with a surfactant, a thickening agent and a sticker (Acronal 4D at 5 % w/v).

This formulation does not mask the repellent activity of cinnamamide which operates through taste or smell in pigeons (Watkins et al., 1994), and the ingredients are inexpensive and environmentally benign. The use of a more effective sticker in the formulation was identified as potentially the most appropriate means of improving the persistence of cinnamamide on oilseed rape. Changing the basis of the formulation from a suspension concentrate to an oil-based or microencapsulated formulation was not considered as these approaches would be likely to mask the primary repellent effects.

Preliminary studies using the original formulation showed that volatilisation and photodegradation would be minimal and that leaching of cinnamamide from leaves would be the major route of loss. The efficacy of the seven alternative stickers below, identified after consultation with commercial producers, was compared with the original sticker, 5 % Acronal 4D in *in vitro* leaching studies. This involved washing the surface of the deposits on glass slides or sprayed leaves with water to simulate rainfall. Studies were carried out to evaluate the various stickers, assess effects of concentration of stickers, the rate of ageing of deposits and effects freezing on the rate of leaching of cinnamamide.

Stickers:
Acronal 4D - a polybutyl acrylate (BASF plc, Cheadle Hulme, Cheshire);
Crovol T40 G, Crovol T60 G (vegetable oil ethoxylates) & Emoleo L1, an activated linseed oil derivative (Croda Chemicals Ltd., Snaith, North Humberside);
Agrimer ST, Agrimer VA6 and Agrimer VA7 W, all vinylpyrrolidone copolymers (ISP Europe, Surrey Research Park, Guildford).

Leaching studies (glass slides)

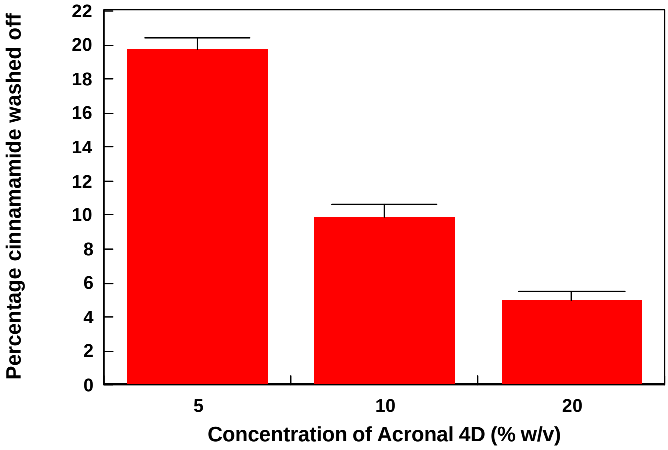
The leaching of cinnamamide was initially assessed by washing dried deposits of various formulations on glass microscope slides with a fixed volume of water using a “rainwashing machine”. This was based on the design used by Phillips (1969) and washes the surface of the slides with a fan-shaped jet of water, adjusted to deliver a volume of 100 ml/minute. This set-up provides a very stringent test of rain-fastness (Phillips & Gillham, 1973).

The formulations were made up to give a concentration of 1 % w/v cinnamamide, by dilution of the suspension concentrate with water. The stickers were also added at the manufacturers recommended rate. 100 µl aliquots of the formulations were pipetted onto glass slides in triplicate, and left to dry overnight, unless otherwise stated. The slides were secured on a vertical plate on the rainwashing machine and then washed with water (100 ml/min) for between 15 and 30 seconds. The water was collected and the volume made up to 100 ml before analysis by UV. The cinnamamide remaining on the slides was also extracted and quantified. From these results, the percentage of cinnamamide washed off each slide was calculated (see Appendix 1 for details of analysis).

Concentration of sticker

Acronal 4D was added to the cinnamamide formulation at 5, 10 and 20 % (w/v). Doubling the concentration of the sticker resulted in the amount of cinnamamide washed off the slides being approximately halved (Figure 2.1). So increasing the concentration of the sticker in the SC formulation would be one method of reducing the leaching of cinnamamide, although it should be noted that high applications of stickers may mask the primary cues of cinnamamide, or may be phytotoxic.

Figure 2.1 Mean percentage of cinnamamide washed off slides with varying concentration of Acronal 4D, after 30 seconds (n=3, ± 1SE)



Ageing of deposits

Slides were prepared using a formulation with Acronal 4D (5 % w/v) as the sticker and were left to dry for either 1, 2, 5, 8, 24 and 48 hours (3 replicates per drying time) before being rainwashed. Residues allowed to dry for 24 hours lost less cinnamamide when washed compared with those dried for shorter periods. There was little difference between slides dried for longer than 24 hours. This implies that in a field situation, a dry period of approximately 24 hours after spraying would be desirable to optimise the adhesion of the formulation with the leaf surface.

Alternative stickers

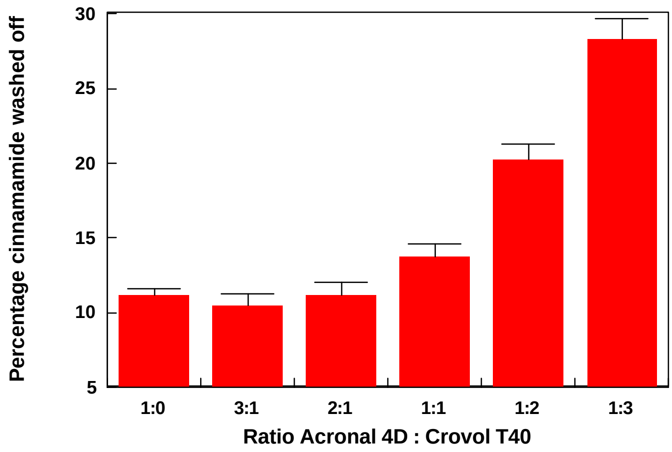
The seven candidate stickers were added to the cinnamamide at the manufacturers recommended rates (5 % w/v for Acronal 4D, Agrimer ST, Agrimer VA6, Agrimer VA7 W and 1 % w/v for Crovol T40 G, Crovol T60 and Emoleo L1). A control formulation without any sticker was also evaluated. As some of the formulations were washed off almost completely after 30 seconds a shorter washing period of 15 seconds was used to compare the stickers. Further formulations were made combining two stickers together, Acronal 4D (5 % w/v) along with each of the other stickers in a ratio 3:1 v/v, at the concentrations above. Deposits of these formulations were also rainwashed (100 ml/min, 20 seconds).

None of the candidate stickers identified showed an improvement on the rainfastness of cinnamamide compared to Acronal 4D. Acronal 4D, a polybutyl acrylate sticker forms a clear film on drying and has excellent "tack" property (Phillips & Gillham, 1973). The three ISP stickers had very little effect on reducing leaching of cinnamamide, whereas the three Croda stickers did lead to a reduction in leaching compared to the formulation with no sticker at all. Crovol T40 G and T60 G are vegetable oil ethoxylates, and form a hydrophobic waxy deposit on drying. Crovol T60 has the higher level of ethoxylates, leading to a higher water solubility, which would explain the lower rainfastness compared to Crovol T40. Emoleo L1 is an activated linseed oil derivative, and forms a hydrophobic film on drying.

Sticker combinations

As the original sticker (5 % w/v Acronal 4D) was found to the best sticker, it was decided to test combinations of the other stickers with Acronal to see if this improved the rainfastness. The addition of Crovol T40, Crovol T60 and Emoleo L1, showed a slight reduction in leaching compared to Acronal alone. This may be ascribed to the combination of the "tack" property of Acronal 4D and the hydrophobic properties of the Crovol T40/T60 and Emoleo L1. The addition of the ISP stickers increased the leaching, so these were not evaluated further. The combination of Acronal and Crovol T40 was further evaluated by changing the ratio of the two stickers (Figure 2.2). It can be seen that there is an optimum ratio of the two stickers (3:1 Acronal: Crovol T40). A higher proportion of Crovol in the formulation leads to increased leaching.

Figure 2.2 Mean percentage of cinnamamide washed off slides with varying ratios of Acronal 4D and Crovol T40 after 20 seconds (n=3, ± 1SE)



Leaching studies (leaves)

In vitro studies were initially performed using deposits on glass slides for reproducibility, due to the heterogeneous nature and complex wetting behaviour of leaf surfaces. Although these studies are very useful for initial screening of stickers, due to the very different nature of the surface of rape leaves and slides, the most promising formulations were also evaluated by washing formulations sprayed onto oilseed rape plants.

Formulations using the most promising stickers, Acronal 4D, Crovol T40, Crovol T60 and Emoleo L1, were sprayed onto growing oilseed rape plants (variety *Apex* 00). Five-week old plants were sprayed with the cinnamamide formulations using an aerosol and left to dry overnight. Leaves (4 replicates per formulation) were then removed from the plants and rainwashed (100 ml/min for 30 seconds), to assess the leaching of cinnamamide. The deposits of cinnamamide remaining on the leaves were extracted and were analysed by HPLC (see Appendix 1).

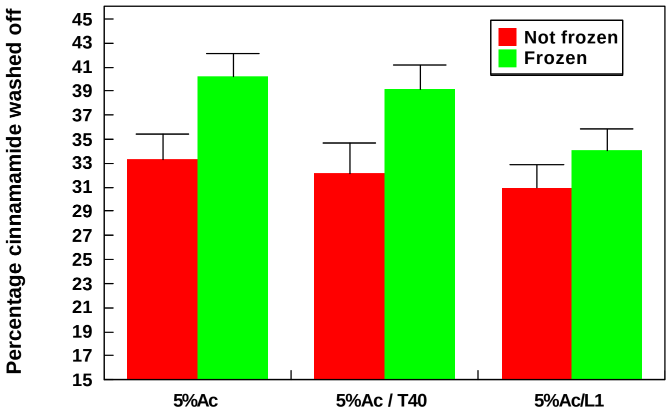
The effect of freezing on the cinnamamide formulations was also investigated, as freezing conditions are likely to be experienced in a field situation. Rape plants were sprayed with formulations made up using the following stickers: Acronal 4D, Acronal / Crovol T40 (3:1 v/v) and Acronal / Emoleo L1 (3:1 v/v). Four leaves were then removed at random from each plant and placed in a freezer for 16 hours. These leaves, along with four leaves from the same plants which had not been frozen, were then rainwashed as before.

None of the candidate stickers showed increased rain-fastness when used alone compared to Acronal, as was found with the deposits on glass slides. However, two of the stickers T40 and L1, when combined with Acronal (ratio 3:1 v/v), did exhibit a reduction in leaching compared to Acronal alone. Doubling the concentration of Acronal 4 D in the formulation resulted in the amount of cinnamamide washed off the leaves being approximately halved.

Effect of freezing leaves

Freezing led to an increase in the amount of cinnamamide washed off the leaves for all of the formulations (Figure 2.3). This may be due to contraction of the sticker on freezing, and on thawing it may become slightly detached from the leaf surface. For the formulation including Emoleo L1, the effects of freezing were much less pronounced than with Acronal alone as the sticker. Hence the addition of Emoleo L1 as well as Acronal to the cinnamamide formulation should be beneficial in the field, as the deposits will invariably be subjected to freeze / thaw cycles during the winter months.

Fig. 2.3 Mean percentage of cinnamamide washed off rape leaves with and without freezing, after 30 seconds (n=4, ± 1SE)



These studies indicated that the persistence of cinnamamide on oilseed rape could be improved either by increasing the concentration of Acronal and / or by using a combination of either T40 or L1 with Acronal 4D (ratio 3:1 v/v). The addition of L1 (and to a lesser extent T40), in the spray mixture was shown to minimise the effects of freezing on the leaching of cinnamamide. It can predicted from these results that using 10 % Acronal / 2 % Emoleo L1 (3:1 v/v) as the sticker, would result in the cinnamamide leached with being reduced by more than half compared to the original formulation.

3. BIOASSAY

Two trials were carried out. Firstly, the optimum application rate of cinnamamide was determined using the original formulation (5 % Acronal 4D as the sticker). Secondly, a trial was carried out to assess whether or not the stickers in the three new formulations, identified above as having improved rainfastness, mask the repellent effect of cinnamamide. Both trials used a two choice test with singly housed feral pigeons. Two trays of rape plants were presented to the pigeons (6 plants per seed tray) for 3 hours each day for 4 days (one tray of plants untreated and the other sprayed with the cinnamamide formulation). After each day the trays of plants were removed and assessed for damage, and a fresh tray introduced the following day. The number of leaves with negligible, moderate and severe damage was recorded, and a damage score calculated for each tray of plants (see Appendix 2).

Optimum application rate

The bioassay was carried out using trays of rape plants sprayed with the original formulation at rates equivalent to 1.25, 2.25, 3 and 4 kg/ha cinnamamide. No signs of phytotoxicity were seen at any of these application rates. The damage scores for the various application rates were calculated and are shown in Table 3.1. At an application rate of 1.25 kg/ha the cinnamamide formulation is clearly not repellent to the pigeons. At 2.25 kg/ha some repellency was shown, however, much stronger repellency was found at 3 kg/ha, with little further increase at 4 kg/ha. Based on these results an application rate of 3 kg/ha was chosen for the bioassay to compare the different formulations and for subsequent field trials. This represented the best compromise between efficacy and economy.

Table 3.1 Mean damage scores for different application rates (mean of 6 pigeons ± 1 SE)

Application rate (kg/ha)	Treated Score	Control Score	Treated/Control Score
1.25	2.39 ± 1.05	2.31 ± 0.87	1.03
2.25	2.29 ± 0.87	2.80 ± 1.76	0.82
3.00	1.94 ± 1.84	2.85 ± 1.90	0.68
4.00	2.01 ± 0.98	3.23 ± 1.26	0.62

Repellency of new formulations compared with the original formulation

The formulations below were used to investigate whether the improved stickers mask the repellent effect of cinnamamide. The design of the trial was based on two 4 by 4 Latin squares, whereby each of the 8 pigeons were presented with each of the formulations over the trial period.

A 5 % Acronal 4 D (original formulation)
B 10 % Acronal 4 D
C 5 % Acronal 4D / L1 3:1
D 5 % Acronal 4D / T40 3:1

The damage scores for the four formulations are shown in Table 3.2. For all formulations less damage was found with the treated than control plants. There was little variation between the four formulations, and no evidence of that the new stickers masked the repellent effects of cinnamamide compared to the original formulation (A).

In the bioassays it was shown that cinnamamide is repellent to feral pigeons at an application rate of 3 kg/ha. A higher loading did not increase the repellency substantially and would be less economically viable. The new stickers identified in the leaching studies do not mask the repellent taste or smell of cinnamamide compared to the original formulation. Doubling the concentration of Acronal has a larger effect on the reduction of leaching of cinnamamide than the addition of L1 or T40 in the mixture. However, in a field situation the rape plants may be subjected to a freeze / thaw cycle, hence the effect of freezing may become very significant. For this reason the addition of L1 to the spray mixture, as well as an increase in the concentration of Acronal would be expected to increase the persistence of cinnamamide when applied under field conditions. The formulation thus selected for use in the field trial was 10 % Acronal 4D / 2% L1 (3:1 v/v).

Table 3.2 Mean damage scores for each formulation (mean of 8 pigeons ± SE)

Formulation	Treated score	Control Score	Treated Score / Control Score
A	1.91 ± 1.23	2.79 ± 1.35	0.68
B	1.67 ± 0.81	2.68 ± 1.04	0.62
C	2.03 ± 1.18	3.23 ± 0.94	0.63
D	1.81 ± 0.81	3.06 ± 0.72	0.59

4. FIELD TRIALS

a) Field trial at Kent 1997/1998

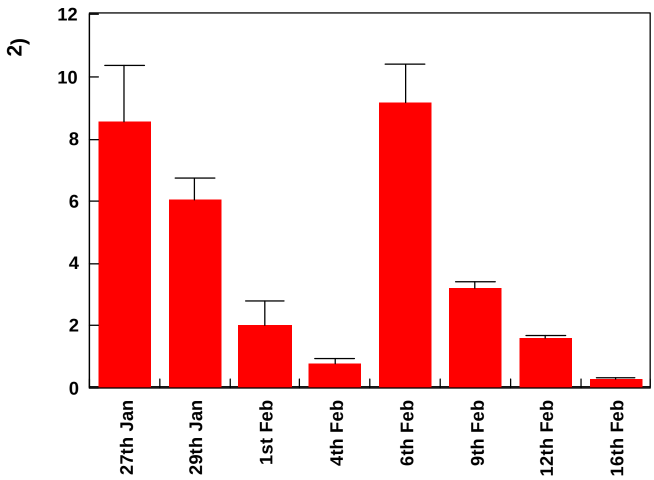
A field trial was carried out on South Darenth Farm, Kent on a field of Apex variety oilseed rape which was sown in September 1997. Twelve 20 × 20 m plots were marked out, with half of the plots sprayed with cinnamamide formulation and half untreated control plots. By the end of January no woodpigeons had been seen in the area. This was probably due to abundant food being available from the good acorn and beech mast crops in the autumn and the very mild winter. However, it was decided to spray the plots with the new formulation in order to determine the persistence of cinnamamide, even if there was to be little woodpigeon damage. The new formulation 10 % Acronal / L1 3:1 was sprayed on the plots at 3 kg/ha on 26th January. A 3600 litre tractor mounted sprayer with a 18 m boom was used. By the beginning of February woodpigeons were seen feeding on the field (after a cold period) and fresh droppings were seen in the plots. Hence a second cinnamamide application was made (February 6th). Woodpigeons were observed on the field site only over approximately a 2 week period (4th February - 19th February) and only in small numbers (less than 200 birds). After this time there were no fresh droppings on the plots and no pigeons were seen in the area. It was also noticed that there were goose droppings in the plots, so some of the damage would also be due to geese.

Leaf samples were taken to determine residues (10 leaves per plot, selected at random by throwing a 40 × 40 cm quadrat) and were frozen until analysis (Appendix 1). Samples were taken the morning after spraying, and 3, 6, and 9 days after spraying. Damage assessments were also made at intervals over the trial and the mean damage score was calculated for the plots (see Appendix 2).

Cinnamamide residues

Two spray applications (3 kg/ha cinnamamide) were made with the following stickers: 10 % Acronal / L1 (26th January) and 10 % Acronal (6th February). The residues of cinnamamide (μg/cm^2 leaf) are shown in Figure 4.1, and the results expressed as a percentage of the initial loading are given in Table 4.1.

Figure 4.1. Cinnamamide residues on leaves (μg/cm^2). Bars represent mean values (n=60 ± 1SE)



The persistence of cinnamamide was increased with both formulations compared to the pilot trial in 1995. In the pilot trial the mean percentage of cinnamamide remaining after 3 and 10 days was 16.2% and 1.9 % respectively. The formulation used in the first spray (Acronal / L1) showed the highest persistence of cinnamamide, as was predicted in the rainwashing studies in the previous reporting period. It should be noted, however, that the persistence is extremely dependent on the weather conditions, so a direct comparison between formulations cannot be made as the formulations were applied at different times.

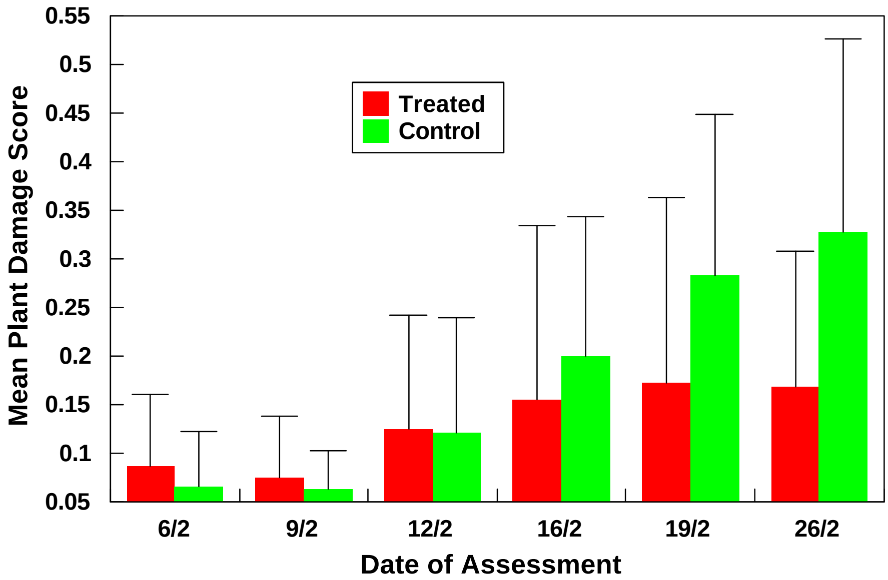
Table 4.1. Mean percentage cinnamamide remaining with time

Time (days)	Percentage remaining after spraying
3	70.6
6	23.7
10	9.3
2	35.2
5	17.3
8	3.8

Woodpigeon damage

There was very limited woodpigeon damage to the plots compared to the pilot trial of 1995. Nevertheless, analyses of the damage assessments were encouraging although the large standard errors reflect considerable variation between plots and, hence, there were no statistically significant differences between treated and control plot means (Figure 4.2). Before the cinnamamide application the treated plots were slightly more damaged than the controls. However, in the period of most woodpigeon damage (between 9 - 19th February) the control plots started to receive more damage, although the results were not statistically significant due to the large variation between plots. By 26th February the woodpigeon damage had stopped.

Fig. 4.2 Mean damage scores for treated and control plots (± 1 SE, n = 6)



b) Field trial at Wintringham, 1998/1999

A field trial was carried out at Shardale Farm, Wintringham, N. Yorkshire to compare the efficacy against pigeon damage, of a cinnamamide application to a standard 00 variety (Apex) with a modified glucosinolate line (JIC 1) – see section II. The field site was surrounded on two sides by woodland and the field has suffered from very heavy pigeon damage over the past few years. Sixteen plots (30 × 24 m) were drilled (8 plots Apex, 8 plots JIC 1) at the end of August 1998, in a chequerboard design. Half of the plots were treated with cinnamamide, and half untreated. This design resulted in four replicates of the four treatments below:

1. Apex + Cinnamamide	2. Apex (untreated)
3. JIC 1 + Cinnamamide	4. JIC 1 (untreated)

First spray: towards the end of January, small numbers of pigeons were seen in the area and slight damage had occurred in adjacent fields. The plots were sprayed (2nd February) with cinnamamide (3 kg/ha) using 10 % Acronal / L1 as the sticker in the formulation. A tractor mounted sprayer with a 24 m boom was used.

Second spray: A very small amount of damage occurred to some of the plots in mid February, so a second application of cinnamamide was made on 18th February (using the same formulation and application rate). Due to problems with spraying using the large scale farm equipment (see below) the cinnamamide formulation was applied to the plots using a knap-sack sprayer.

Unfortunately problems were experienced during the first spray application with the farm equipment. It was found that on spraying the cinnamamide formulation, the filters in the sprayer had become clogged with a white rubbery substance. This is thought to be due to some reaction, possibly polymerisation of the Acronal in the tank mixture. The exact cause of this is not known - no problems have been encountered when using knapsack or aerosol sprayers, and we have not been able to reproduce any similar reaction of Acronal in the formulation in the laboratory. It is possible that it may be due to the high aeration / pressure of the spray mixture whilst being constantly re-circulated through the system when the tank mixer is switched on before the spraying even commenced. It may be that if the tank agitation is switched off that this problem would be overcome (the motion of the tractor should prevent the cinnamamide from settling out at the bottom of the tank), although further work would be needed to confirm this.

Cinnamamide residues:

Leaf samples were taken repeatedly after spraying to determine changes with time in cinnamamide residues on the two rape varieties (10 leaves sampled per plot which were selected at random by throwing a 40 × 40 cm quadrat) and were frozen until analysis by HPLC. These analyses showed that the persistence of cinnamamide had again been greatly improved compared to the pilot trial (where residues had fallen to approximately 15 % after 3 days and 2 % by 10 days) by using the modified sticker in the formulation (Figure 4.3 and Table 4.2).

Woodpigeon damage:

Despite large flocks of woodpigeons being observed in the area, the level of damage to the field site was very disappointing. A very small amount of damage first occurred to some of the plots in mid-February. This was following a period of a few days when the plots were completely covered in snow, and cinnamamide levels after the first spray were very low by this stage. Only half of the plots received any damage at all, and those which did only suffered negligible damage to a few plants. The damage was scored using the same method as in previous years, however no significant differences between the treatments were seen due to the low baseline level of damage. The mean score maxima for this year were 0.05 and 0.08 for treated and untreated plots respectively, compared to the previous year's mean score maxima of 0.17 and 0.32 (treated and untreated plots).

Figure 4.3. Cinnamamide residues on leaves (µg/cm ^{2}). Bars represent mean values (n=40 ± 1 SE)

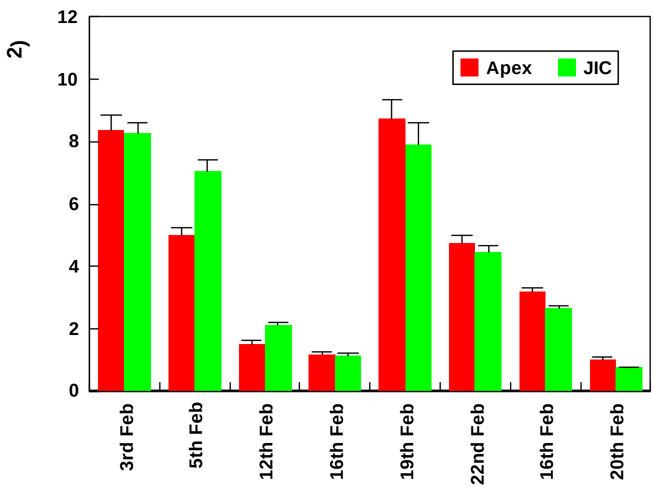


Table 4.2. Mean percentage cinnamamide remaining on Apex and JIC 1 lines

Spray	Time (days)	% Cinnamamide remaining (Apex)	% Cinnamamide remaining (JIC 1)
1st spray	3 10 14	59.8 18.2 14.0	85.1 25.8 13.9
2nd spray	4 8 12	54.4 36.8 11.7	56.5 33.8 9.7

Yield:

The final yield was assessed for the Apex and JIC 1 line plots, with and without cinnamamide application. It can be seen (Table 4.3) that the cinnamamide sprayed plots had a slightly, but non-significantly, higher yield than the untreated plots. It was encouraging that there is no apparent yield penalty or phytotoxicity with the application of cinnamamide.

Table 4.3. Mean yield (tonnes per hectare) for each of the four plot types (n=3, ± 1 SE)

Treatment	Mean yield (t/ha)
Apex	3.53 (0.12)
Apex + cinnamamide	3.57 (0.09)
JIC 1	3.57 (0.12)
JIC 1 + cinnamamide	3.73 (0.09)

c) Field trial at Wintringham 1999/2000

Due to the lack of pigeon damage to the field site the year before, the project was extended to enable a satisfactory assessment of the efficacy of the improved cinnamamide formulation and a comparison with the JIC unpalatable line of rape. To maximise the likelihood of getting pigeon damage on the plots, two fields with historically high pigeon pressure on a large arable farm set amongst extensive mixed woodland were selected for the trial. A total of 16 plots (20 by 25m) of the 4 treatments below were drilled in two separate fields (4 replicates per field) at G & H Chomley, Wintringham. Blocks of the 4 treatments "quadruple plot design" were spaced over 2 fields to maximise the likelihood of pigeon damage on the plots:

1. Apex (no cinnamamide)
2. Apex + cinnamamide
3. JIC (no cinnamamide)
4. JIC + cinnamamide.

A field visit in early November revealed that in one field, (*field 1*), the growth of the crop was noticeably poor, with very small plants and numerous bare patches. The second field (*field 2*) was better established, with much larger plants. Large flocks of woodpigeons were noted in the area (> 500) and by early December there was a significant amount of pigeon damage occurring in *field 1*, which continued over a 2 month period. There was also some damage to *field 2* by the middle of January, but this was on a smaller scale and only occurred for approximately 2 weeks. Fortunately the significant amount of damage to the field site this year has allowed for a good assessment of the efficacy of cinnamamide and the JIC high glucosinolate line of rape to be made. The more substantial and longer period of damage to *field 1* is examined in more detail below.

Field 1

By early December large numbers of pigeons were seen in the area and significant damage was occurring. The plots were sprayed with cinnamamide (3 kg/ha) using the improved formulation (10 % Acronal / L1 as the sticker). Three spray applications were made using a knap-sack sprayer, on 13th December, 17th January and 17th February.

Damage assessments of the plots were made on a regular basis from mid December to mid March (see Appendix 2). This enabled thorough monitoring of changes in levels of damage with time to assess the efficacy of the cinnamamide spray and the JIC line of rape. Leaf samples were also taken throughout the trial (10 leaves per plot, selected at random by throwing a 40 × 40 cm quadrat) and were frozen till analysis (Appendix 1).

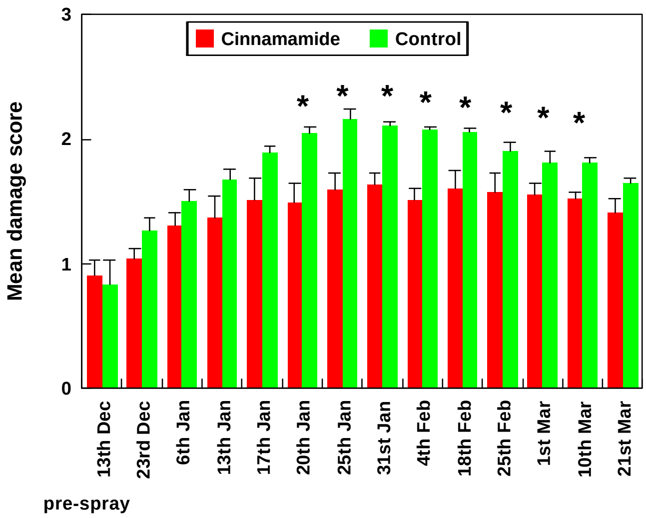
Efficacy of cinnamamide:

The mean damage scores for Apex plots with and without cinnamamide are shown in Figure 4.4. The results are very encouraging. It can be seen that there was already significant pigeon damage by the time the plots were first sprayed (13th December). Most of the damage to treated plots occurred between 23rd December and 17th

January, when cinnamamide residues were low (see Figure 4.9). This was during a long period of snow / sleet and heavy winds which prevented a second spray being made until 17th January. The largest differences between control and treated plots can be seen towards the end of January, when treated plots had only 73 % the damage of un-sprayed plots. The damage to cinnamamide plots was statistically significantly less than the control plots from 20th January till 10th March (P<0.05). For the week following the second spray (17th Jan - 25th January), when cinnamamide residues were high, the damage score for control plots increased from 1.89 to 2.16 (a 14 % increase), whereas there was a much smaller increase in the damage score of 6 % for treated plots (from 1.51 to 1.60).

It should be noted that towards the end of the assessment period, the mean damage scores for all plot types start to decrease. This can be ascribed to fresh growth of the leaves during a period of rapid growth (the damage scores are based on the proportion of the plant which is damaged, so as new leaves grow and old damaged ones die off, then the damage score will fall).

Figure 4.4 Mean damage scores for cinnamamide treated and untreated Apex plots (n=4 ± 1SE, * P < 0.05)



The mean damage score provides a summary of the damage, based on the proportions of undamaged plants and those with negligible, moderate and severe damage (see Appendix 2). Figures 4.5 to 4.7 show the proportion of plants damaged within each class at three intervals over the trial - before spraying, in the middle and near the end of the trial. It is very encouraging to note that there was a significant reduction in the proportions of plants with severe damage in cinnamamide treated plots (P < 0.05 for 20th January and 18th February), with a higher proportion of plant with no or negligible damage. It is the severely damaged plants, particularly if the inner growth point is damaged, which are less likely to recover and which may represent substantial reductions in yield.

Figure 4.5 Proportion of plants in each damage class for treated and control Apex plots on 13th December before spraying (n=4 ± 1SE)

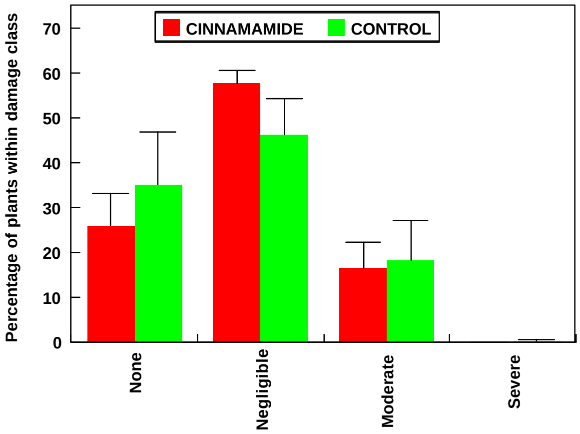


Figure 4.6 Proportion of plants in each damage class for treated and control Apex plots on 20th Jan (n=4 ± 1SE, * P <0.05)

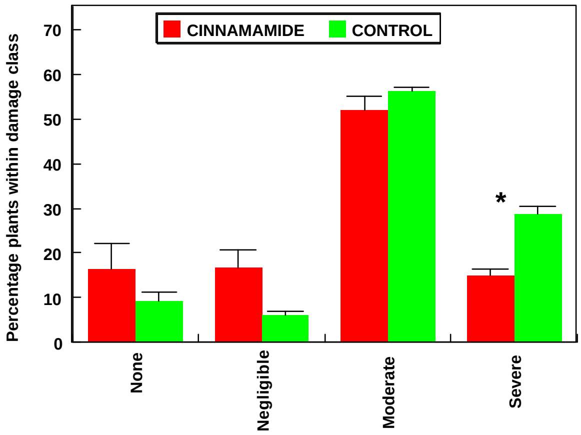
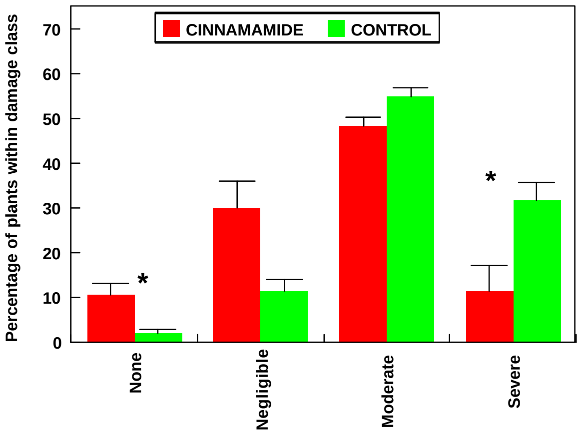
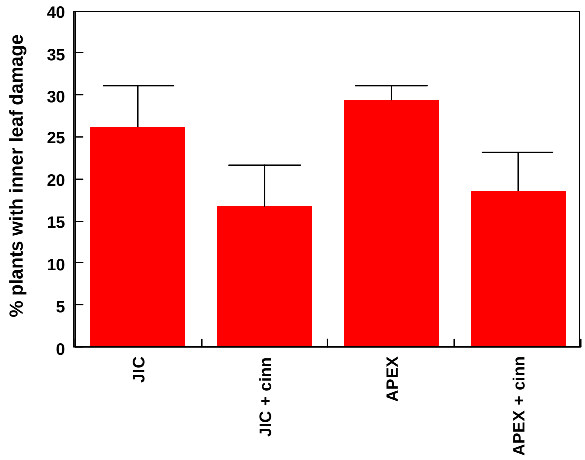


Figure 4.7 Proportion of plants in each damage class for treated and control Apex plots on 8th Feb (n=4 ± 1SE, * P <0.05)



The proportion of plants with their inner leaves damaged was also assessed, and is shown for all four plot types in Figure 4.8. The cinnamamide treated plots had significantly fewer plants with inner leaf damage than unsprayed plots. For both Apex and JIC plots the differences between sprayed and unsprayed plots were statistically significant ($P < 0.05$). It was also observed that the JIC line of rape had fewer plants with damaged inner leaves compared to Apex, although this was not statistically significant.

Figure 4.8 Percentage of plants with inner leaf damage, 25th Feb (n=4 ± 1SE)



Cinnamamide residues:

Leaf samples were taken at regular intervals during the trial in order to determine the residues of cinnamamide on the leaves of both Apex and JIC rape (see Appendix 1). Again, the persistence of cinnamamide was found to be greatly increased using the improved formulation compared to the pilot trial (where residues had fallen to 15 % after only 3 days). It should be noted that there was a long period (between 23rd December and 17th January) when the residues were very low, whilst pigeon damage was occurring.

Figure 4.9 Cinnamamide residues, µg/cm ^{2} leaf (n= 40 ± 1SE)

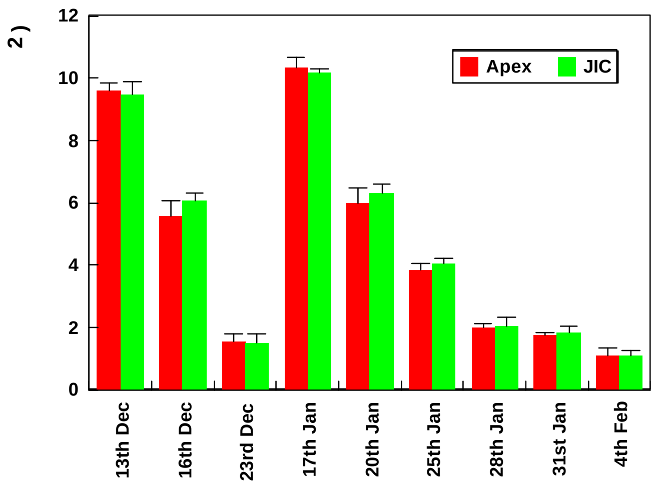


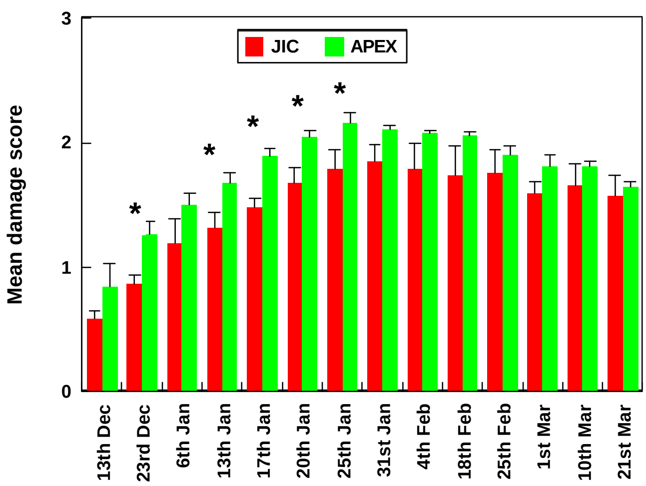
Table 4.4 Percentage cinnamamide remaining after spraying

Time (days)	Apex	JIC
Spray 1		
3 days	58.1	63.8
10 days	16.1	15.8
Spary2		
3 days	58.0	62.0
8 days	37.2	39.6
11 days	19.4	20.1
14 days	16.9	17.9
18 days	10.7	10.8

JIC versus Apex:

The mean damage scores for Apex and the high glucosinolate JIC plots (without cinnamamide application) are shown in Figure 4.10. It can be seen that the JIC plots were less damaged than the Apex plots, particularly early in the trial period (results were statistically significant between 23rd December and 25th January). The largest differences between the plots was found in December, when the JIC plots had 68-70 % the damage of the Apex plots, whereas later in the season the differences were reduced (JIC plots had approximately 80 % and 85 % the damage of Apex in mid-January and mid-February respectively).

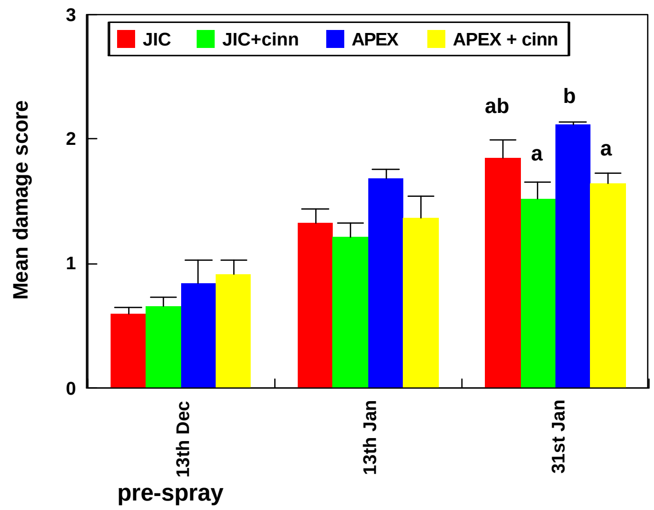
Figure 4.10 Mean damage score for unsprayed Apex and JIC plots (n = 4 ± 1SE; * $P < 0.05$)



Combination of cinnamamide and high glucosinolate rape:

The mean damage scores for all four plot types, Apex and JIC with and without cinnamamide are shown in Figure 4.11. The least damaged plots were the plots with the combined "double protection" of the JIC rape and the cinnamamide spray. By the end of January, cinnamamide sprayed JIC plots had only 70 % the damage of unsprayed Apex plots. A one-way ANOVA showed that the difference in damage between the 4 plot types were significant by 31st January, and post-hoc comparisons revealed that the JIC plots with cinnamamide had significantly less damage than unsprayed Apex plots ($P < 0.05$).

Figure 4.11 Mean damage score for all 4 plot types (nb. 13th Dec. pre-spraying)



Field 2

There was no damage at the beginning of January on this field, but by the 18th January there was substantial damage. The plots were sprayed with cinnamamide on the 19th January, but as can be seen from Figure 4.12 there was only a small amount of damage after this, so no information regarding the effectiveness of cinnamamide can be gained from this field. As the JIC plots had their high glucosinolate protection in their leaves when the pigeon damage occurred, this field produced useful data for comparing JIC and Apex (Figure 4.13). The results look promising, particularly early in the season, when the JIC plots had at their best only 63 % the damage of the Apex plots, although due to the variability between plots the differences were only significant on the 28th January assessment.

Figure 4.12 Mean damage scores for Apex plots with and without cinnamamide (n=4 ± 1SE)

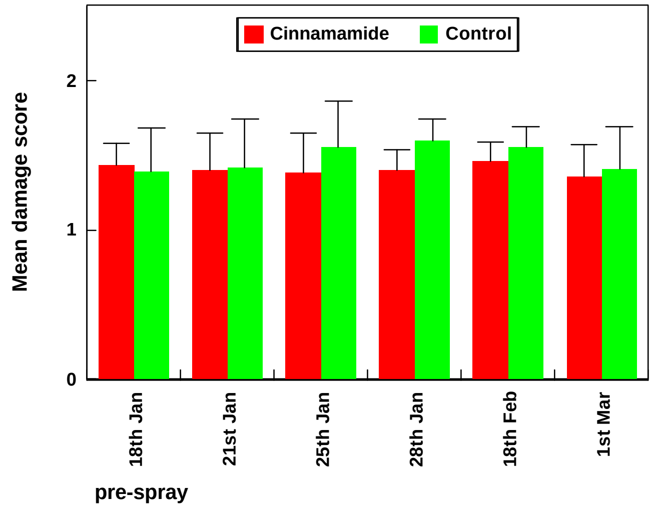
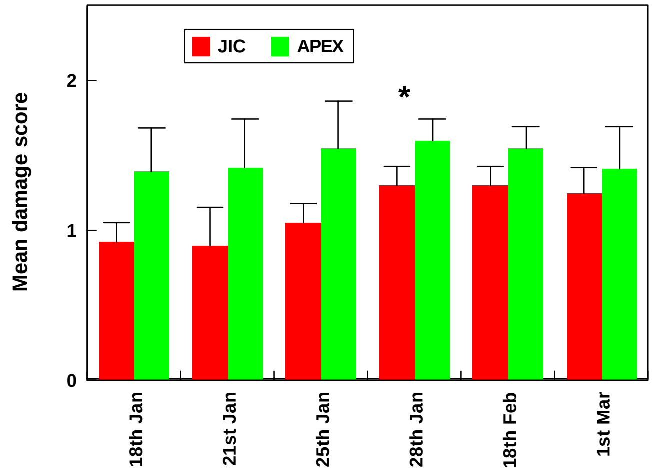


Figure 4.13 Mean damage scores for Apex and JIC plots (n=4 ± 1SE, * P < 0.05)



Yield:

The plots from both fields were taken on to harvest to determine the yield. The mean yield of all the plots was 3.02 ± 0.11 tonnes per hectare (n=30, ± SE), with no statistically significant differences between the various plot types. It is disappointing that the reduction in pigeon damage on the cinnamamide treated plots and JIC plots was not reflected in higher yields. This may be due in part to the fact that the cinnamamide was not applied until there was already a significant amount of damage to the plots. If it had been present over the duration of the period when damage was occurring it is more likely that a yield increase would have resulted from spraying with cinnamamide. The results may also reflect the ability of the crop to recover from a certain amount pigeon damage without significant yield losses, and of course there are many factors other than pigeon damage which have an impact on final yield.

5. General Discussion

A more weather-resistant suspension concentrate (SC) formulation of the avian repellent cinnamamide has been developed. It was predicted that the leaching of cinnamamide from this improved formulation, which uses a 10 % Acronal / 2 % Emoleo L1 mix in the ratio 3:1 v/v as the sticker, would be less than half that of the original formulation (5 % Acronal as the sticker). The inclusion of Emoleo L1 in the formulation is beneficial as it reduces the effects of freezing on the rate of leaching. The bioassay using captive pigeons shows that, at an application rate of 3 kg/ha, cinnamamide is an effective feeding deterrent for pigeons. Furthermore, these studies have demonstrated that the modified stickers do not mask the repellent properties of cinnamamide. Hence the improved formulation was suitable for use in subsequent field trials.

The field trials carried out over three consecutive years confirm that the persistence of cinnamamide has been greatly improved in the modified formulation. The cinnamamide residues found in these studies 3 days of application were between 58 and 71 % of the original levels, with approximately 15 % remaining after a period of 10 days. This enhanced persistence of the improved formulation is encouraging, considering the harsh winter conditions at the time of spraying, and the fact that the topical application is inevitably ` the growth of the plant and the emergence of new leaves.

In the 1999/2000 field trial there was sufficient woodpigeon damage to the field site to assess the effectiveness of the improved cinnamamide formulation and compare it with the high glucosinolate JIC line of rape. Cinnamamide was shown to reduce the level of pigeon damage to rape, and towards the end of January treated plots had only 73 % of the damage of control plots. Although the cinnamamide did not keep the pigeons off the plots altogether, there were significant reductions in the number of plants which were severely damaged. It is these severely damaged plants which are least likely to recover, leading to constraints on yield. The JIC rape was also shown to be less palatable to pigeons, particularly early in the trial period. Plots with the “double protection” of the cinnamamide spray and the high glucosinolate rape were the least damaged. By the end of January, plots of the JIC rape which were also sprayed with cinnamamide, had only 70 % the damage of untreated Apex plots.

Limitations of cinnamamide as a feeding deterrent:

The cinnamamide formulation is designed as a topical foliar application to protect the rape. As such there will be inherent limitations on persistence due to the growth of leaves which dilutes the application, and as old leaves die back the compound is lost. This means that in practice more than one application would be required to protect the rape during its vulnerable growth period. Furthermore, poor weather may restrict access to fields with sprayer equipment during the winter, when the pigeons are most likely to be causing damage. This is one area where systemic approaches may be more practical, or as with the JIC cultivar, unpalatable cues is built into the rape.

Benefits of cinnamamide as a feeding deterrent:

We have shown in this project that when cinnamamide is present on the leaves, it is effective at reducing the amount of severe pigeon damage. It could be used to protect a whole field of rape, or it may be more practical and economical to spray onto areas of the crop more prone to damage eg. headlands, where crop is slow to get established, under pigeon flight lines, and areas surrounded by trees where pigeons roost. The JIC line of rape was found to be less palatable to pigeons than Apex, however, later in the growing season in February and March, this effect was less marked. Cinnamamide could be sprayed onto JIC rape at this stage, to provide an extension of the period of protection from pigeons, which can be quite severe at this time of year as the crop takes off and fresh succulent growth is present.

Cinnamamide is not yet registered under the Control of Pesticides Regulations for use as a repellent on oilseed rape. Industrial sponsorship is currently being pursued to take it through to the registration process. This research, which has shown cinnamamide to be effective in reducing damage to rape by a major pest species, the woodpigeon, should enhance the prospects of gaining such sponsorship. Other applications for the compound may include the protection of other vulnerable crops from pigeons such as linseed and peas, and in the horticultural field transplanted brassica plants, and utilising its repellent effects against slugs, and its suitability to be incorporated into seed dressings.

Cinnamamide is currently an expensive compound because it is not manufactured in significant quantities. However, it is a simple compound to produce and should it become commercially available, bulk synthesis from readily available inexpensive precursors would be viable. As such, it is hoped that ultimately cinnamamide offers the prospect of an effective and environmentally benign means of reducing woodpigeon damage oilseed rape.

II. BREEDING HIGH GLUCOSINOLATE VARIETIES

The effects of changing leaf GSL profile on pigeon feeding on oilseed rape

1. INTRODUCTION

The woodpigeon, *Columba palumbus*, is a major pest of winter oilseed rape. Woodpigeon numbers have increased in the last decade, possibly due to relatively mild winters and the availability of winter food sources such as fields of winter oilseed rape. Pigeons start flocking at the onset of cold weather, usually in mid November, and it is these flocks which do most of the damage to rape fields (Murton & Jones 1973).

The damage that these birds cause may not lead to yield loss if there is only light grazing, as the plants can compensate for some leaf area loss, but in severe cases, the whole field may have to be ploughed in and re-sown with a new crop in the spring. The variability in damage between years and sites and the unpredictability of these flocks makes pigeons a very difficult pest to control (Murton & Jones 1973). Attempts at reduction in pigeon numbers by shooting have been shown not to reduce flock size or damage to crops (Murton *et al*. 1974) and therefore crop protection is seen as the best way to reduce damage (Hunter 1974). The various scarer mechanisms are often useless after only a few days of use as the birds become used to them (Davis 1974), and the options of sowing early or over-fertilising the plants to make the field less attractive to pigeons can be unacceptably costly if there is not guaranteed to be damage over the winter.

As generalist pests have been shown to be deterred by increased levels of glucosinolates (GSLs) in the leaves of *B.napus* (Giamoustaris & Mithen 1995), the idea of breeding agronomically acceptable lines of oilseed rape with elevated or altered GSL levels to deter these birds is attractive. These lines would offer a low-maintenance insurance mechanism for farmers as they would have built-in defences against the pigeons.

This section describes the effect of lines of oilseed rape with altered leaf GSL profiles on the feeding of pigeons. The complexity of analysing the pigeons' responses to these lines and aspects of experimental design are discussed.

2. METHODS

a) Development of OSR lines with altered leaf glucosinolates

The OSR lines used in this study were derived from wild *Brassica* diploid species. Initially, a novel amphidiploid *Brassica napus,* of genome AACC was developed by the hybridisation of a wild accession of *B*.

rapa (genome AA) and a wild form of *B. oleracea* (genome CC). These plants were selected as they possessed GSL profiles distinct from those found in standard oilseed rape varieties. The *B. rapa* plant originated in Sicily and contained high levels of but-3-enyl GSL, and the *B. oleracea* plant chosen was *B. oleracea* var. *atlantica* from Tunisia, which had very high levels of prop-2-enyl GSL in its leaves and seeds (Giamoustaris 1995).

The F_1 hybrid between these lines was produced by ovary culture and embryo rescue, and then colchicine was used to double chromosome numbers to produce the synthetic amphidiploid plant SYN292 as described in Mithen and Magrath (1992). This plant was produced in 1991 and became part of a breeding program to introgress genes responsible for novel GSL profiles into agronomically acceptable lines of oilseed rape. The double low winter oilseed rape line Cobra was used as a recurrent parent in this program. SYN292 was crossed to Cobra, and a selected F_1 individual was backcrossed to Cobra to produce 100 B_1 individuals. Glucosinolates were analysed from these and a self-compatible line producing prop-2-enyl GSL, as well as a GSL spectrum which suggested heterozygosity at several GSL loci, was selected and selfed. Eighty B_1S_1 individuals were grown in glasshouse conditions and seed and leaf GSL analysis performed on them. One line with high levels of prop-2-enyl and but-3-enyl GSL in its leaves was then crossed to a breeding line from CPB-Twyfords Ltd (CPB5071) which had low levels of hydroxylated GSLs in its leaves and low total seed GSLs. The new F_1 hybrids produced were selfed and the F_2 seedlngs hardened off in cold frames and transplanted into netted field plots in Norwich in March 1994. These individual plants were bagged, the seed collected and analysed for GSL content and 11 individuals with <20 μmol/gDW total seed GSL were selected. These were planted out in a replicated field experiment in the autumn of 1994 and one line AGF32, with high leaf GSL and low seed GSL as well as high resistance to *P. brassicae* and *L. maculans* was selected for further crosses.

An individual from AGF32 was selfed, and from field evaluation of the progeny, three individuals were selected and selfed to produce the experimental lines, F3210, AGF32-34 and F3237. These were crossed once more to line CPB5071. Pollen from one selected F1 plant from each line was microspore cultured (as described in Magrath *et al.* 1993) and the resulting lines colchicine doubled to produce Doubled-Haploid (DH) seed in September 1996.

Forty-two lines were produced from the AGF32-10 parent (the F3210 population), 43 from the AGF32-37 parent (the F3237 population) and 2 from the AGF32-34 parent. This breeding program is summarised in Figure 2.1.

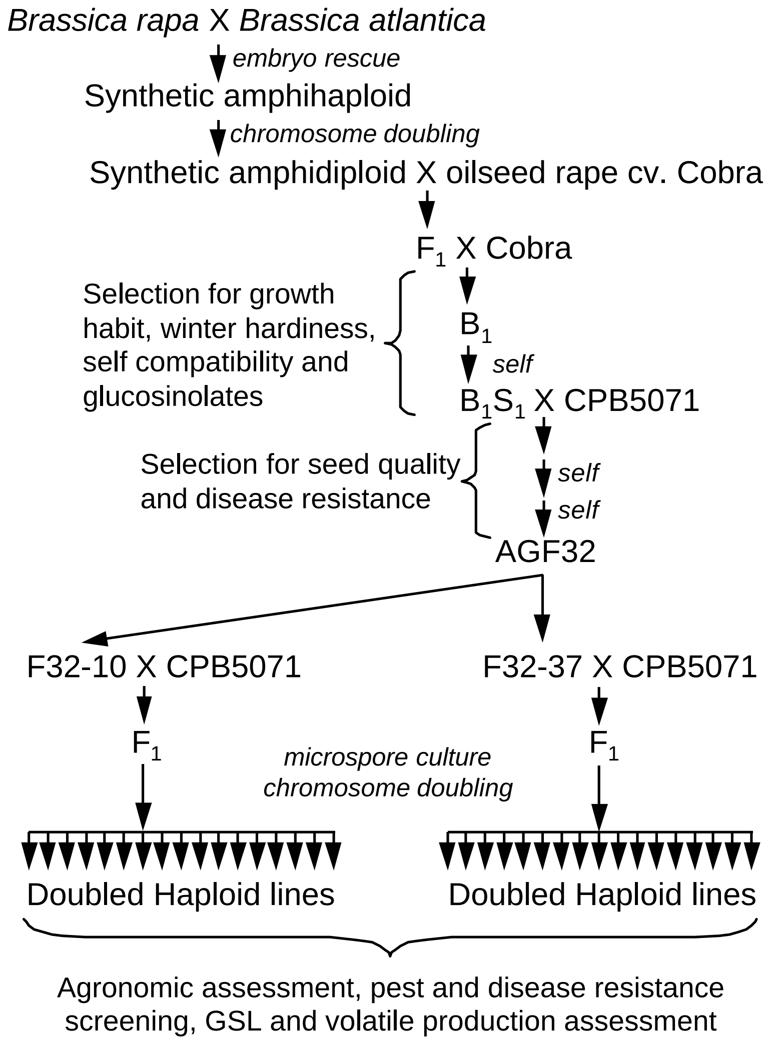


Figure 2.1. Breeding programme summary.

Seed from each of these lines was germinated in the glasshouse and harvested for DNA extraction. Fifty of the remaining plants were hardened off in the cold frames and ten were transplanted to pots in either an unheated, unlit glasshouse, or a heated lit glasshouse. All of these plants were vernalised in short day vernalisation rooms held at 4°C for 8 weeks during December and January before returning them to their respective glasshouses. The remaining thirty plants were used as part of the field trials for 1996/7.

1996/7 field trials

A trial to assess pigeon damage on experimental lines was established at Churchdown Farm in Kent, in September 1996. This consisted of four blocks of Apex and four blocks of selfed seed from the parental line F3210 in a checkerboard design with squares 40 m x 40 m of each variety. These were grown in the same way as standard winter oilseed rape throughout the year. Unfortunately there was contamination of the seed drill for two of plots, so assessments were done on only 3 pairs of plots. A pigeon damage assessment was done in January and GSL analysis was also performed at this time. The plots were harvested by combine-harvesting the central 30 m x 30 m square from each of the plots for a yield assessment in July 1997. To score the plots for pigeon damage, a transect line was taken through the centre of each plot and all plants touching that line (approximately 90 in each plot) were scored for the number of leaves that showed signs of pigeon damage. Thirty leaves from each of the plots were also removed and frozen in solid CO_2 for GSL analysis (see 2.2).

1997/8 field trials

Two field trials were conducted in this growing year. Due to self-incompatibility of some lines, 75 lines were used in a field trial conducted at Yarmouth farm, Lulworth, Cambridgeshire. 2 m x 10 m plots were drilled at a rate of 120 seeds/m^2 on the 30th August and 3rd September in a completely randomised design on a flat area of the field. They were separated by 0.5 m on their short axis and 2 m on their long axis. Wherever possible, three replicates of each line were drilled, although there was only enough seed in some cases to drill one or two plots. AGF32-37 (F3237), F3210, CPB, Cobra, Apex, Nickel, Pronto, and Synergie were planted out as part of this design as controls.

Plants were analysed for leaf GSLs on December 22nd and 23rd. They were also scored for flowering time in March/April, leaf colour in January, and height and lodging in June. In March it became apparent that there was a high incidence of volunteer oilseed rape in some of the plots and therefore all the plots were rogued by hand to remove as many of these as possible.

Three of the highest yielding lines from the 1996/7 field trial which also produced good GSL spectra in their leaves were planted in a separate field trial in Otley, Suffolk. 4 m x 15 m blocks were drilled at 90 sd/m^2 with 0.5 m between each block. The blocks were sown in a chequerboard design, alternating each DH line with a block of Apex. Leaf GSLs were collected from these lines every 6-8 weeks for analysis of changes through the year, and several assessments of pest damage were scored on these plots throughout the year. The field trial in Otley, was scored for hare and pigeon damage on the 13th November when the plants were approximately at the 12 leaf stage and again on the 12th December. Two transects were taken through the plots, each 4m from the end of the plots. One was scored on each date to avoid resampling. All plants touching the line were scored (approximately 30 plants for each plot) for the number of leaves pecked. Note was also made of any patchiness and of specific damage patterns in the November sample. GSL samples were taken at both sampling times as for the Kent field trial.

1998/1999 trials

Two lines were chosen from the previous year's field trials which showed the most promising agronomic characteristics and GSL profiles. These were F3237-115 (JIC1) and F3237-205 (JIC2). The trials were run by Velcourt U.K. at three sites, Bishop Fonthill in Dorset, West Winch near Kings-Lynn, Norfolk, and Wintringham in Yorkshire. A checkerboard design was drilled at 120 seeds / m^2 in blocks 20 x 30 m. Each block was split in half for fungicide treatments and at the Wintringham site an extra six blocks were sown, three to be sprayed with cinnamamide (see section I). Apex was used as a control at all sites with Cobra and CPB5071 being used as additional controls at the West Winch site.

1999/2000 field trials

A number of fields known to be affected by pigeon damage were sown with either pure F3237 115 or a mixture of this with other double low cultivars. No quantitative scores were performed, but reports from the farm managers have been collated to assess the effectiveness of these lines in large scale trials. A similar trial to those in Dorset and Yorkshire in 1998/9 was run by CPB-Twyfords this year at a site in Cambridgeshire. Again this was only scored visually.

b) Glucosinolate analysis

<u>Preparation of tissue for analysis</u>

Seeds used for analysis were air-dried on the plants, threshed and cleaned of all unwanted material. The water content of seeds dried in this way is approx. 6% (measured by NIR spectroscopy). Seeds were ground in a coffee grinder for 30 seconds immediately prior to extraction to minimise glucosinolate breakdown by endogenous myrosinase activity.

Leaf tissue collected from glasshouse grown plants was immediately placed in liquid nitrogen after removal from the plant. Tissue collected from the field was frozen in solid carbon dioxide for transport back to the laboratory. The tissue was then freeze-dried for at least four days. The tissue was ground to a fine powder and stored inside sealed plastic bags with silica gel at -20°C until needed for analysis.

Glucosinolate extraction

This was done by conversion of the glucosinolates to desulphoglucosinolates (DSGSL) and then eluting them from an ion exchange column as described in Heaney *et al.* (1986). Between 300 and 400 mg ground tissue was placed in a 50 ml centrifuge tube. 10 ml hot 70% methanol was added and then 100μl 16mM glucotropaeolin (benzyl glucosinolate) was added as an internal standard. The tubes were then vortexed and incubated at 70°C for twenty minutes, vortexing andother two times during the incubation. The tubes were then centrifuged at 3000 rpm for 5 minutes.

A 0.5 ml column of DEAE Sephadex A25 (Pharmacia) (in 0.02M sodium acetate) was washed twice with 0.5 ml distilled water (dH_2O) and then 3 ml of the supernatant were added. Once this had dripped through the column, it was washed twice with 0.5 ml H_2O and twice with 0.5 ml 0.02M sodium acetate. 75μl purified sulphatase (arylsulphite sulphohydrolase SIGMA S-9626 EC 3.1.6.1) at approx 3 units/l were added and the columns were incubated for 16 hours at room temperature. The desulphoglucosinolates (DSGSL) produced were then eluted in 1.25 ml dH_2O and stored for analysis at -20°C.

Separation and quantification of desulphoglucosinolates.

DSGSLs were separated on a reverse phase HPLC column (spherisorb 5*m* ODS2 250 x 4.6 mm cartridge, Anachem) using the following acetonitrile / water gradient:

0 mins	1% acetonitrile
23 mins	20% acetonitrile
31 mins	20% acetonitrile
35 mins	1% acetonitrile
40 mins	1% acetonitrile

The DSGSLs were detected by their absorption at 229nm and identified with reference to standards identified in Heaney *et al.* (1986). Quantification was through comparison with the internal standard, using the response factors as published by Haughn *et al.* (1991) and Heaney *et al.* (1986). Their concentration in μmol/gDW was calculated by the following equation:

$$\text{GSL concentration} = \frac{\mu\text{mols internal standard x response factor x peak area}}{\text{Standard peak area x weight of sample}}$$

3. Results

1996/7 field trial

Only three of the pairs of plots from this trial could be analysed due to contamination of the seed drill in the sowing the final F3210 plot. The GSLs from F3210 and Apex lines showed significant differences (Figure 3.1).

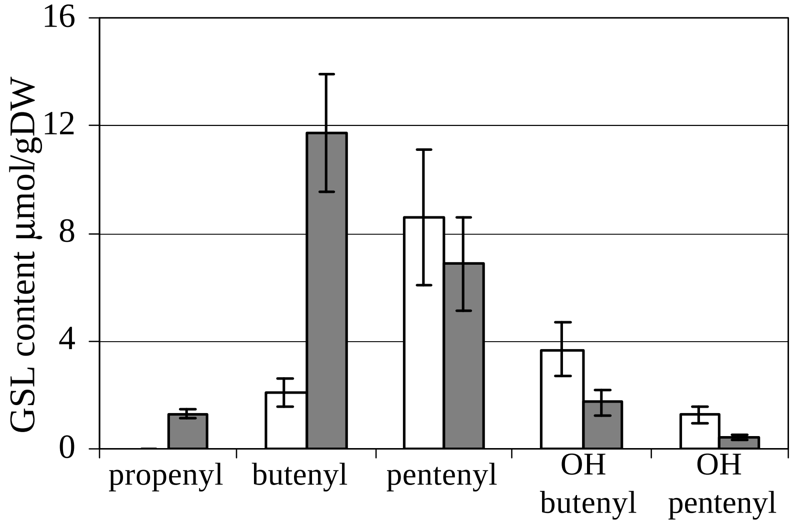


Figure 3.1. GSL profiles from the F3210 (grey bars) and Apex (white bars) plots in Kent. Results are given as the mean of four replicates ± 1 s.d.

The levels of pigeon grazing were not high on any of the plots. The damage was greatest on the plots furthest from the woodland where the plants were noticeably smaller than those in the plots nearest the woodland (5-8). There was a significant reduction in pigeon damage in two of the pairs of plots (5&6, 3&4) shown by Mann Whitney U tests (the data were not distributed normally). In plots 7 and 8 however, damage was too low for there to be any significant differences seen between the plots (figure 3.2). In plots 7 and 8 the plants were of the greatest size, and comparison of the two F3210 plots and two Apex plots nearest the woodland showed a significant reduction in damage in plots 7 and 8 (Apex 5 > Apex 7 $p < 0.0005$, F3210 6 > F3210 8 $p = 0.011$). Pigeon damage was also noticeably greater on sparser areas of the field than on the more dense areas.

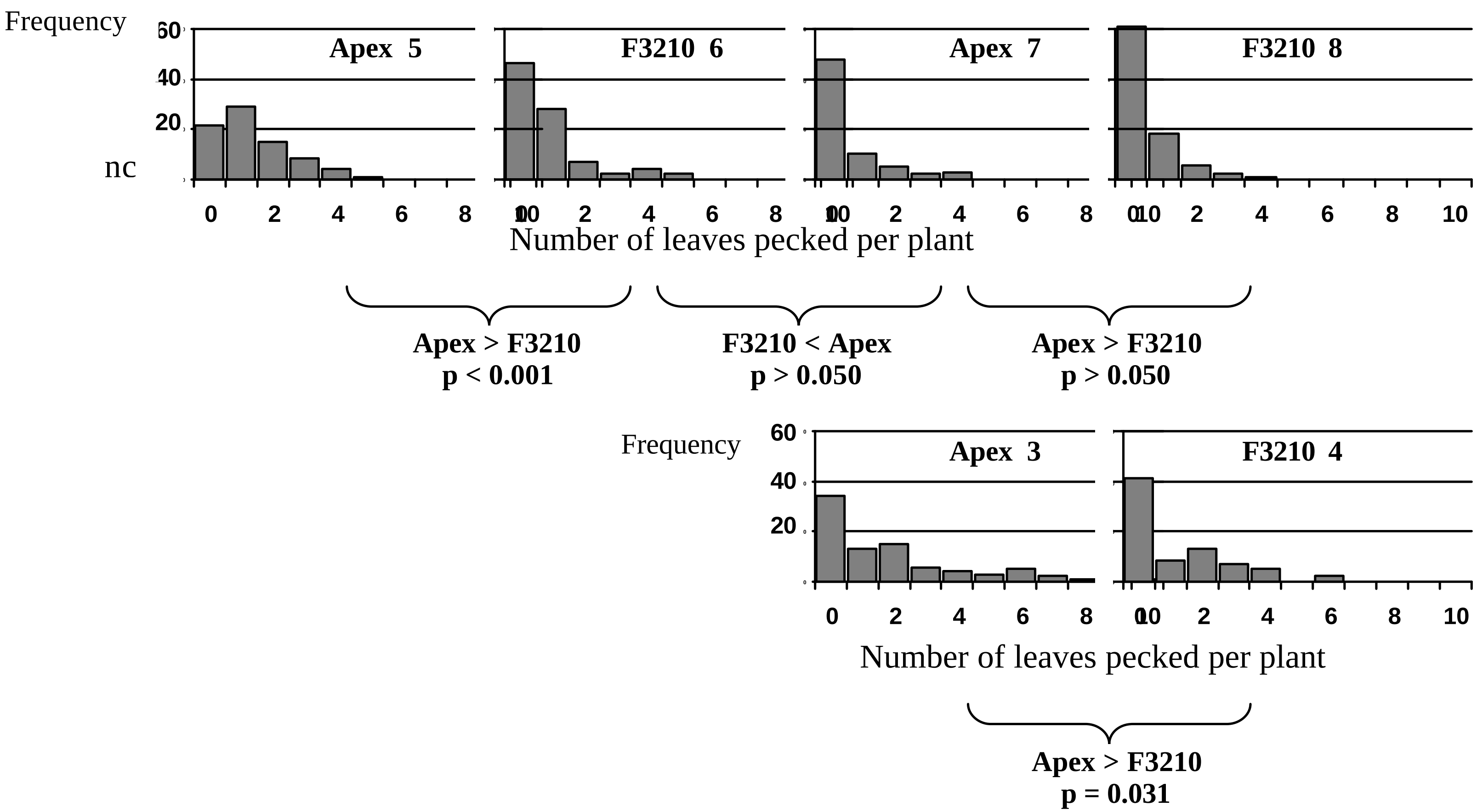


Figure 3.2 Pigeon damage on the Kent field trial. p values are given from Mann Whitney U tests between pairs of plots as the levels of damage varied so greatly across the field.

1997-98, Otley field trial.

There was damage on these plots starting early on in the Autumn before pigeons had started flocking in East Anglia. Further damage was also seen in December when pigeons had been seen at the site. However, at both times, damage was extremely light, with only one or two leaves on each plant being damaged in many cases. The damage done to each of the lines is shown in figure 3.3.

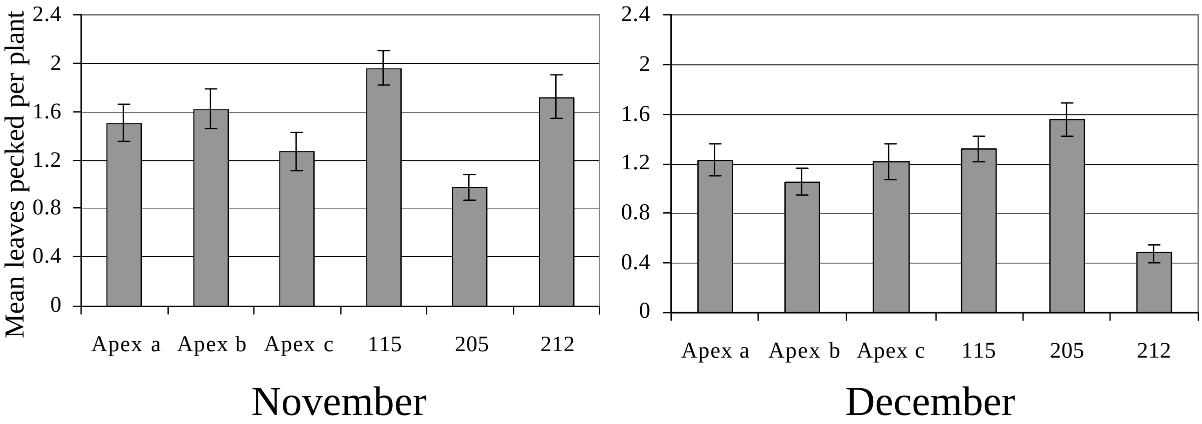


Figure 3.3. Damage (mean ± 1 s.d.) done to plants at the Ipswich field trial in November and December.

In November the F3237 205 line was the least damaged with the F3237 115 line being slightly more damaged than the Apex controls. In F3237 115 plot 1 which was nearest to the woodland, there was quite extensive grazing in one corner of the plot which might account for the increase in damage seen on this line. Without this one plot, the mean damage falls to 1.23 pecks per plant, which is not significantly different from that found in the Apex control plots (ANOVA $F = 0.23$, $p = 0.64$). However, in December, the F3210 212 line showed much less damage than any of the other lines, with F3237 205 being slightly more damaged than the Apex controls.

Field trials in 1998/9.

There was no significant pigeon damage reported on any of the field trials at any of the sites in this year. However, in the absence of pigeon grazing, the two lines chosen, F3237 115 and F3237 205, both showed good yields in comparison with Apex (Figure 3.4), F3237 115 yielding not significantly less than Apex at any site.

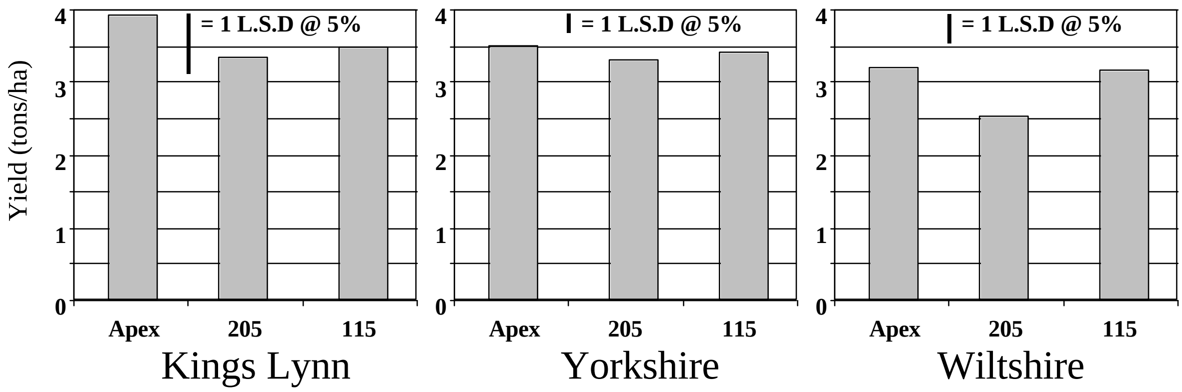


Figure 3.4. Yield from field plots in 1998/9 field trials. Figures are given as means of the replicate plots with the Least Significant Difference between plots at 5% significance shown.

Field Trials 1999/2000.

In these large-scale field trials, no quantitative assessment was done on pigeon damage, but damage was observed on several sites where the line F3237 115 had been planted. A summary of the reports from the sites which have suffered significant pigeon grazing is given below.

Goodleys – Kings Lynn, Norfolk: Admixture field appears to have reduced pigeon grazing compared with non-admix fields.

Chomley – Malton, North Yorkshire: 1st field later drilled, grazed by pheasants and pigeons, no difference between Apex and F3237 115. Madrigal, a faster growing variety, is showing less damage. 2nd field is much further ahead in growth stage and showing much less pigeon grazing. Here in areas where pigeons have been grazing there is slightly more grazing on Apex then F3237 115.

Haverholme Park – Lincolnshire: One field with pure F3237 115 planted is surrounded by fields with other double low varieties. There is no pigeon grazing on the F3237 115 field but all other fields are under high grazing pressure. Confirmed by R.F.Mithen (pers. comm.).

Cornbury – Cotswolds: Several fields with F3237 115 mixed into double low varieties. Any fields with the mixtures are experiencing very low levels of pigeon grazing compared to surrounding fields without the mixtures.

Hinchwick – Cotswolds: One field planted with an admixture. This field is untouched by pigeons, other fields surrounding are being attacked.

CPB-Twyfords trial: The field where the F3237 115 was planted in blocks with Apex has been badly grazed by pigeons since November and there appears to be no difference between the two varieties.

4. Discussion

The results from the four years of field trials have shown the difficulty of establishing good a relationship between a plant's putative defences and the actions of a generalist herbivore. With pigeons, it seems that there are a number of factors which must be considered when attempting to deter them from fields. Factors which have been reported to influence pigeon feeding behaviour and crop damage are summarised in table 4.1. All of these factors (except for scarers, which were not employed at any site) were varying in the different field trials, and they probably each contributed to the differences seen between trial sites and lines of *B.napus*.

Table 4.1. Factors which influence pigeon feeding and crop damage.

Factor	**Effect of factor**	**Reference**
Weather conditions	Early cold periods can cause damage to start earlier, and cold snaps in the spring can cause it to continue longer then usual	Jones (1974) Murton & Jones (1973)
Field site	Quiet fields near woodlands most severely attacked	Jones (1974) Murton & Jones (1973)
Surrounding fields	Availability of preferred food sources elsewhere can delay damage to fields	Jones (1974) Murton & Jones (1973)
Plant size	Small plants most severely attacked	Jones (1974)
Plant spacing	Widely spaced plants offer more visibility and are more attacked	Jones (1974)
Scarers	If regularly changed can reduce damage, but birds often become habituated to them	Hunter (1974) Davis (1974)
Plant taste	GSL increase decreases damage, as does application of Cinnamamide	Gill *et al.* (1998) Giamoustaris & Mithen (1995)

Weather conditions

The low levels of damage reported in the years 1996-1999 were possibly due to the mild winters of those years. The weather was especially mild in the early spring, when pigeon damage has been reported to be at its most severe (Murton & Jones 1973). This possibly could have resulted in other food sources, such as clover, becoming available to the pigeons, so reducing their numbers on the trial sites (Murton *et al.* 1974). However, in the last field trial in 2000, the winter appears to have been hard enough for considerable damage to be done to the sites. This unpredictability in pigeon numbers and feeding habits is one of the biggest problems in assessing pigeon damage and control methods (Hunter 1974).

The effect of field site and trial size

Regular human disturbance at the field site has been shown to offer the best protection against pigeon damage in the past (Jones 1974). Therefore all field sites in the present study were chosen to be close to woodland (where the woodpigeons can roost) and in secluded locations. The sites were all known to be very badly affected by pigeons in previous years, and yet were only very lightly attacked in the years under study. This again highlights the variability in feeding damage over different years and the subsequent difficulty with assessing control methods.

In addition it appears that positioning of the trial plots is important. In the Dorset field trial (1998/9), pigeon grazing was extensive on the field nearest the woodland, but was negligible where the trial plots were planted. Conversely, in the Ipswich field site, the F3237 115 plot planted nearest the field border was the only one to be heavily damaged, which skewed the results for that line (see above). This represents a further problem with the study of crop protection from pigeons. Although in previous studies small 12 x 2 plots have given good clear differences between lines (Giamostaris and Mithen 1995), in that study there were 100-fold differences in GSL levels between lines tested and therefore the difference in taste to the pigeons would probably have been immediate enough for them to choose between lines in small plots. There was very little difference in total aliphatic GSL levels between lines tested in the present study, especially between Apex and F3237 115. Therefore the repellent effects were likely to have been smaller, so requiring a larger experiment to reduce the obscuring effects of patchy feeding over the site. Previous studies on pigeon protection methods have looked at areas no smaller than whole fields (Murton *et al.* 1974, Murton & Jones 1973) and it seems likely that assessment of any repellent effect of the DH lines will have to conducted on this scale to give a reliable result.

<u>The effect of surrounding fields</u>

In studies comparing damage on Brussels sprouts and cabbages, Jones (1974) showed that the pigeons preferentially grazed the Brussels sprouts and did not damage the cabbage field until the sprouts had been harvested. Therefore it seems that if there are more attractive options available in other fields, pigeons will move on from the less attractive crop. This may account for some of the low damage recorded in the present study as all trials were planted in areas surrounded by oilseed rape fields which potentially provided a more attractive feeding area than the trial plots. For example, in the 2000 trials, at four out of six sites there was high grazing pressure on surrounding fields but very little on the trial field, suggesting that the pigeons were avoiding it in favour of the standard lines of oilseed rape.

Even if the whole area were planted with a resistant variety, allowing the pigeons no choice of food source, there may still be benefits to growing less palatable varieties. If these more resistant varieties succeed in moving the flocks of pigeons from field to field in search of more palatable lines, the redistribution of damage could be sufficient to prevent any one field becoming damaged past the level from which the plants can recover without yield loss (Murton & Jones 1973). Due to the difficulty in predicting pigeon damage and the lack of other methods of control, this may provide the most satisfactory solution for farmers.

<u>Plant size and spacing</u>

Jones (1974) found that small plants were attacked in preference to large ones, possibly due to the pigeons' like for being able to see in all directions when feeding. Sparse areas of the fields studied were preferentially attacked, possibly for the same reason. Both the Kent trial (1996/7) and two of the trials in

2000 support this observation. In Kent, there was a clear increase in damage on the plots which had smaller plants, regardless of the genotype (figure 3.2). The only two trials in 2000 which showed no difference between F3237 115 and Apex were those which had small plants. In both cases this was due to very early (October-November) grazing on the plots keeping the plants small before aliphatic GSL production had increased. In the Chomley site the effect of plant size was shown even more clearly as not only did the faster growing variety Madrigal suffer less damage on the same field, but another field further ahead in growth stage also showed far less damage and in this case there were differences observed between the two Apex and F3237 115.

<u>Plant taste</u>

With so many other factors playing a role in determining final pigeon damage to a crop it is impossible to say for certain which is the most important, however, evidence from the four years of field trials does suggest that the altered taste of the DH lines is having some repellent effect on pigeons.

Kent field trial

The F3210 line grown in Kent had leaf GSLs significantly higher in total than the Apex control and suffered less damage in the worst affected plots, as suggested by previous studies (Giamoustaris & Mithen 1995). It also had much higher proportions of the shorter chain, non-hydroxylated GSLs, but-3-enyl and prop-2-enyl GSL, which may have contributed to this repellent effect (Giamoustaris & Mithen 1995). As Gill *et al.*(1998) have shown that pigeons make food choices based on taste alone, it is assumed that the pigeons are avoiding these GSLs due to taste rather than any toxic effects which they might suffer from ingesting them (Griffiths *et al*. 1998).

Ipswich field trial

The 1997/8 trial in Otley, Ipswich also showed significant reduction in grazing on two of the DH lines. In November this was the F3237 205 line and in December this was the F3210 212 line. All four lines were producing both GSLs and volatile breakdown products at these two times, and therefore the results seem inconsistent, especially as at both of these time periods the F3210 212 line was producing at least five times the levels of but-3-enyl that the other DH lines were producing. If the reduction in damage were solely due to GSL content or volatile production, then one would expect the reduction in damage to be greatest on this line at both time periods.

However, in November, hare droppings found in the plots suggested that the damage was not done by pigeons. Line F3237 205 produces rather flat rosettes with many more trichomes on the upper surface than Apex of the other two DH lines in this trial. This makes the plants very spiky to the touch and this is likely to have been an added deterrent to the hares. In December, these trichomes offered no extra resistance to the beaks of the pigeons which were now grazing the plot, especially as the newer winter leaves were less hairy than the autumn rosette. Therefore at this time the resistance of the F3210 212 line may truly have been due to the high levels of GSL-derived volatiles that it was producing. However, this line was also larger than the other two lines at this stage in the winter and therefore physically may not have made such an attractive feeding ground for the pigeons (Jones 1974).

To support the argument that it is the GSLs which are acting as a deterrent, it was noticed that there were differences in the style of feeding damage between Apex and this line. In Apex, the pigeons appeared to have concentrated on the central regions of the plants, possibly where the leaves were softer and more nutritious. By contrast, in the F3210 212 line, the only leaves that had been pecked were the very outermost leaves of each rosette. It is known that GSLs are higher in younger leaves of *B. napus* (Li *et al.* 1999b) and this may have provided enough deterrent to prevent the pigeons from pecking at these leaves. This protection of the apical meristem could be important in reducing yield loss as it would allow earlier flowering by the plants, making the field less acceptable to pigeons and therefore shortening the damage season. Unfortunately the F3210 212 line did not yield as well as the other two DH lines in this trial or the Cambridge field trial and therefore this effect could not be followed up in the subsequent year’s field trial.

1999/2000 field trials

The 1999/2000 trial results showed significant effects of pigeon damage at several sites. At many of the sites where a pure field or a mixture of F3237 115 has been drilled, there appears to be less grazing damage, although on surrounding fields there is significant pigeon damage. The effect of mixtures is particularly interesting as it suggests that if indeed the pigeons are being deterred by the bad taste of the F3237 115 plants, they do not need to taste every plant before giving up and choosing another field. This is possibly because pigeons are known to eat a little of many plants in the field, rather than concentrating on one (Murton & Jones 1973), and therefore they would quickly encounter the F3237 115 plants in a mixture. Using mixtures could provide a way to introduce these pigeon resistant lines into mainstream farming more quickly than trying to breed all of the GSL traits into the latest high yielding lines, as the yield loss that might be incurred by growing these plants would be lessened.

In conclusion it seems that the introgression of novel alleles into winter oilseed rape lines which alter the GSL profiles in their leaves may indeed offer increased resistance to pigeon grazing as long as there is not too much damage done to the plants early on in the winter. However, the decrease in pigeon damage is unlikely to be of economic value. It is therefore concluded that the development of OSR lines with enhanced glucosinolates should be seen as one component in the fight against pigeon damage.

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Appendix 1: Analytical methods (I, trials with cinnamamide)

Leaching studies

In the studies to assess the leaching of cinnamamide from various formulations applied to glass slides or rape leaves, the cinnamamide remaining was extracted as follows:

Slides: after rainwashing the slides were allowed to dry overnight. Cinnamamide was extracted from the slides with methanol (5 × 1 ml) into a small beaker. It was possible to scrape the sticker film from the slides using a microspatula. The slides were then washed with a further 2 ml methanol. The extracts were sonicated for 10 minutes and then left to stand for 2 hours. The extract was then filtered and transferred to a volumetric flask, with washings. The volume was made up to 10 ml and then diluted to 10 % before analysing by UV spectrometry.

Leaves: after rainwashing the leaves were allowed to dry for at least 2 hours. Cinnamamide was extracted from the leaves by sonicating twice for 10 minutes in 10 ml of solvent (methanol / water 4:1 v/v). The extracts were decanted and combined and the volume made up to 25 ml. Samples were filtered (syringe filter, Whatman, 0.45 µm) before analysis by HPLC.

The water washed over the residues in the leaching studies was collected, the volume made up to 100 ml and analysed by UV spectrometry.

UV analysis

A Shimadzu UV - visible spectrophotometer (UV-160A) was used for the analysis of aqueous solutions of cinnamamide from the rainwashing studies using glass slides and leaves, and for the methanolic extracts of cinnamamide remaining on the slides after washing. Samples were filtered (syringe filter, Whatman, 0.45 µm). Calibration curves were obtained using standard solutions (between 1 and 15 mg/l) of cinnamamide in both water and methanol. Analysis was at a wavelength, λ max of 276 nm and 272.8 nm respectively.

HPLC

A Waters-Millipore HPLC system was used for the analysis of methanolic solutions from plant extracts. A Kromasil RP-18 (5 µm) analytical column was used (250 × 4.6 mm) with an injection volume of 100 µl injection volume, and detection at 280 nm. The mobile phase consisted of A, potassium dihydrogen orthophosphate buffer (pH 7.0, 20mM) and B, methanol. A gradient profile was used at a flow rate of 1.0 ml/min: 0-2 mins 30 % B; 2-8 mins 30 - 90 % B; 8-13 mins 90 % B, 13-15 mins 90 - 30 % B; 15-17 mins 30 % B. Calibration curves were obtained using standards of cinnamamide in the range 0.5 to 20 mg/l (in methanol / water 4:1 v/v).

Field Trials

Leaf extraction:

Cinnamamide was extracted from the leaves by sonicating each leaf twice for 10 minutes in 10 ml of solvent (methanol - water 4:1 by volume). The extracts were decanted and combined in a volumetric flask, and made up to 25 ml. Samples were filtered (syringe filter, Whatman, 0.45 µm) before analysis using HPLC as above. The residues were calculated as the amount of cinnamamide per area of leaf (µg/cm^2).

Appendix 2: Damage assessments (I, trials with cinnamamide)

Bioassay :

The six plants in each tray were assessed for damage by the pigeons as follows: the number of leaves on each plant were counted, and then the damage to each leaf was classified as either “negligible” (a few pecks from the outer lamina), “moderate” (up to 50 % of leaf area taken), or “severe” (more than 50 % of leaf area taken), it was noted whether or not the inner growth point was damaged for each plant.

An overall damage “score” was calculated for each tray of plants:

= (no. leaves with negligible damage) + (2 × no. leaves with moderate damage) +
(3 × no. leaves with severe damage) / total no. of leaves

Field trials:

Damage assessments were made at intervals throughout the three field trials. For each plot, all of the plants within 15 randomly positioned 40 × 40 cm quadrats were assessed. As the numbers of plants involved in the damage assessments in the field were significantly larger than in the bioassay, the assessments were done on a plant basis rather than going down to the detail of numbers of leaves. The number of plants within the quadrat were counted, and then the damage to each plant was classified as either “negligible” (a few pecks from the outer lamina of leaves), “moderate” (up to 50 % of leaf area taken), or “severe” (more than 50 % of leaf area taken); it was also noted whether or not the inner growth point was damaged for each plant.

To simplify the damage, a **“damage score”** was calculated for each plot:

= (no. plants with negligible damage) + (2 × no. plants with moderate damage) +
(3 × no. plants with severe damage) / total no. of plants

Statistical analysis:

Means of the damage data were calculated for the replicate plots of the field trials for each treatment. Damage data was then compared between treatments by paired t-test or using a one-way ANOVA.