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OAK PARK

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Research Report

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Director

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Crops Research Centre
Oak Park, Carlow
Telephone: (059) 9170200 Fax: (059) 9142423

Staff

(as of 31 December 2009)

Prof. J.I. Burke, B.Agr.Sc., M.Sc., Ph.D., Head of Centre

Crop Science Department

P.D. Forristal[5], B.Agr.Sc. *(Acting Head)*
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G.O'Dea[2], B.Sc.,
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B Yang[2] B.Sc M. Sc., Ph.D.
R. Shinde[2] B.Sc (Hons), PhD
S. Moreria[2] PhD
S. Morris[2] B.Sc (Hons), PhD

***Technicians**:* O. Aylesbury, P. Brett, B. Burke, J. Connery, K . Crombie J. Deegan, J. Dillon[1], J.Grace, F Hutton, A. Mahon, , K Murphy, H. O'Shea, P. Prior, P. Tiernan[1], E.Young[3], T. Ralph

Plant Sciences Biotechnology

S. Barth, Ph.D.
D. Milbourne, Ph.D.
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T.Murray[3, 4] Ph.D
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S.Phelan[3]. B.Sc. Ph.D
I. Nagy[3] PhD
G.Bigio[2] M.Sc.
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***Technicians**:* T. O'Connell (Stores)
J.J. Hogan (Farm Manager)

Executive Assistants: C.Austin
E.Butler
S.Cosgrove
P.Kelly

[1] Retired 2009
[2] Walsh Fellow
[3] Contract Staff
[4] Contract Ended
[5] Acting Head
[6] Career Break

Fungicide resistance in cereal pathogens

O'Sullivan, E. and Kildea, S. **RMIS No. 5764**

Strobilurin resistance in *Mycosphaerella graminicola*

Leaf blotch caused by *Mycosphaerella graminicola* (*Septoria tritici*) is the most important foliar disease of wheat in Ireland. Failure to control this disease would lead to drastic reduction in wheat yields and wheat-growing would become uneconomical. The use of fungicides is therefore essential to producing economically sustainable yields. During 2002 resistance to the strobilurin (QoI) group of fungicides emerged in *M. graminicola* in Ireland and by the end of 2003 strobilurin resistance was endemic in populations of the pathogen in all wheat crops, usually at frequencies approaching 100%. There followed a dramatic reduction in the use of strobilurin fungicides in disease control programmes thus reducing the selection pressure for resistance. Studies on the resistance of *M. graminicola* populations in winter wheat crops to strobilurin (QoI) fungicides have been ongoing since 2003 and were continued in 2009. The objective was to determine if, with the reduction in the selection pressure resulting from a reduction in the use of strobilurins, resistance levels would decline over time.

Leaf samples infected with *M. graminicola* were collected from 10 crops selected at random in the main winter wheat-growing regions of the country in July. Monopycnidial isolates of *M. graminicola* were obtained from 20 randomly-selected leaves from each crop and grown on potato dextrose agar (PDA) for four days until conidia were produced. Conidial suspensions of each isolate were added to potato dextrose broth (PDB) in microtitre plate wells in increasing concentrations (0, 0.04, 0.37, 0.12, 1.1, 3.3, 10 and 30 µg ml^{-1}) of technical grades azoxystrobin. Salicylhydroxamic acid (SHAM) (40 µg ml^{-1}) was added to the PDB to inhibit an alternative oxidase respiratory pathway in *M. graminicola*. Growth was assessed as a measure of optical density at 405 nm using a Tecan Saffire II plate reader following 10 days incubation at 18^0C. Growth of sensitive isolates was inhibited at fungicide concentrations greater than 0.12 µg ml^{-1} azoxystrobin while resistant isolates grew well at concentrations up to and including 30 µg ml^{-1} of the fungicide. High levels of strobilurin resistance were again detected in populations of *M. graminicola* in wheat crops. Resistant isolates occurred with frequencies of 100% in 9 of the 10 crops sampled and at 50% in the other crop. The average was 95% for the 10 crops studied. The continuing high levels of resistance, following seven seasons of reduced use of strobilurin fungicides, indicate that the G143A point mutation encoding strobilurin resistance in *M. graminicola* is genetically stable, does nor carry a fitness penalty and will continue to remain high regardless of selection pressure.

Sensitivity of *Mycosphaerella graminicola* to triazole fungicides

The development of resistance to strobilurins in 2003 meant that control of *M. graminicola* would, for the immediate future, be almost entirely dependent on the triazole group of fungicides. It was feared that the selection pressure imposed on the pathogen by the more intensive use of these fungicides might lead to the development of resistance or reductions in sensitivity that would affect their field performance. Populations of *M. graminicola* in randomly-selected wheat crops in Ireland have been studied since 2004 for sensitivity to four commonly-used triazole fungicides, epoxiconazole, prothioconazole, tebuconazole and metconazole.

Samples of infected leaves were collected in March, to determine the levels of sensitivity at the beginning of the season, and again in July following completion of the fungicide control programmes. Isolates of *M. graminicola* (20 per crop) were obtained, tested for growth in technical grade fungicide concentrations (0, 0.04, 0.37, 0.12, 1.1, 3.3, 10 and 30µg ml^{-1}) and growth assessed as already described. EC50 values (concentrations at which growth was inhibited by 50% relative to untreated controls) for each fungicide were determined for all isolates.

There was a shift in sensitivity to tebuconazole and metconazole between 2004 and 2005. There was cross-sensitivity between both fungicides i.e. isolates of *M. graminicola* with reduced sensitivity to tebuconazole were also less sensitive to metconazole (see Research Report for 2005). *M. graminicola* populations have remained stable since then at the levels of sensitivity detected in 2005.

Studies on the sensitivity of *M. graminicola* to epoxiconazole and prothioconazole showed sensitive and stable populations up to spring 2008, all isolates having EC50 values of less than 1µg ml^{-1} (LogEC50<0µg ml^{-1}) for both fungicides (Figures 1 and 2) However, in summer 2008 a shift in sensitivity to epoxiconazole and prothioconazole was detected in one of the wheat crops sampled. Isolates of *M. graminicola* were found in that crop which were less sensitive to these two fungicides, particularly to prothioconazole (EC50>10µg ml^{-1}, LogEC50>1µg ml^{-1}), than any detected previously and they comprised the majority of the pathogen population in the crop. These insensitive strains were found in a wheat crop in the same field in April 2009, before there was any further selection pressure from fungicides and at the same high frequency as in the previous summer. This suggested that there was unlikely to be a fitness penalty associated with them. Investigations of *M. graminicola* populations in other crops in spring 2009 showed a general shift in sensitivity to both epoxiconazole and prothioconazole (Figures 1 and 2). The majority of isolates from the shifted populations remained at the least sensitive end of the range of sensitivities detected previously. However, more insensitive isolates, similar to those detected in the one crop in 2008 occurred at frequencies of 5% to 10% in all crops. Investigations of *M. graminicola* populations in summer 2009 confirmed the shifts in sensitivity detected in spring. The consequences if any of this shift in sensitivity will be studied over the coming seasons.

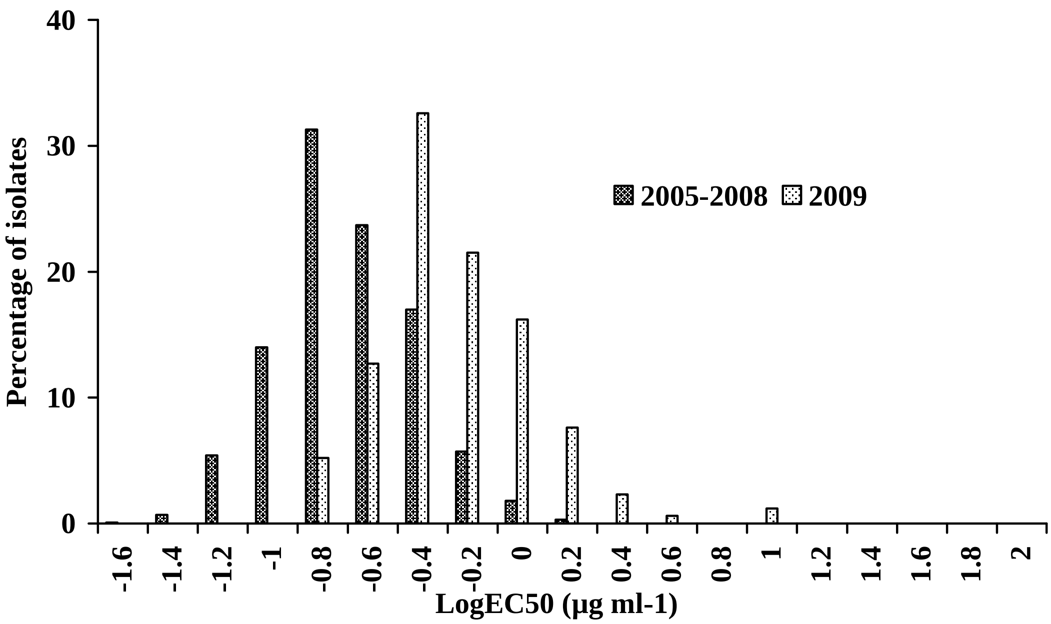


Figure 1: Sensitivity of Mycosphaerella graminicola isolates to epoxiconazole (Opus (Opus) 2003 - 2005

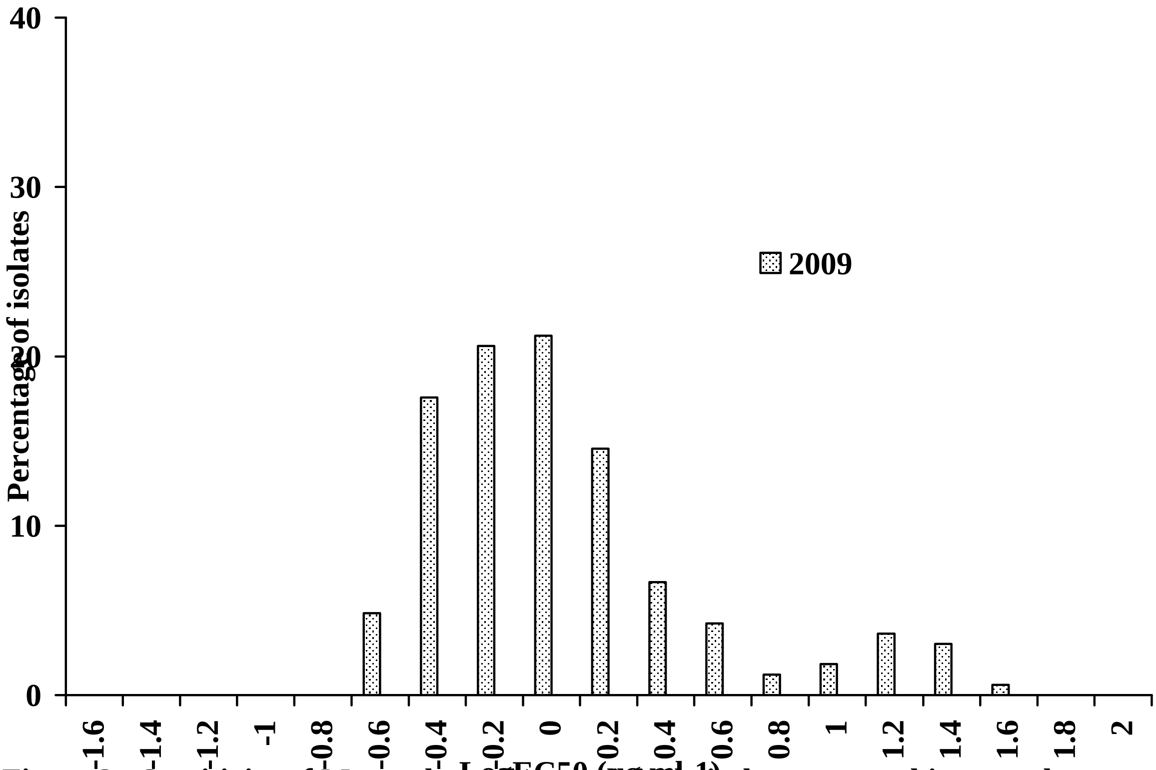


Figure 2: Sensitivity of Mycosphaerella graminicola isolates to prothioconazole

Mutations and resistance groups in *M. graminicola*.

The pressure imposed on *M. graminicola* by the intensive use of triazole fungicides over several decades has led to genetic changes or mutations in the gene (CYP51) encoding the target site for these fungicides. This results in amino acid substitutions in the target protein which may interfere with the binding of the fungicides and result in resistance or reduction in sensitivity. Isolates of *M. graminicola* have been classified into seven resistance groups, R1 – R7 (Leroux *et al*., 2007) based on mutations in the CYP51 gene and their effects on sensitivity. Sequencing analyses of over 200 isolates from the 2006 and 2007 Irish *M. graminicola* populations identified a number of different mutations (see Research Report for 2007). The majority of isolates (c70%) were classified as R5 with the remaining 30% distribute within R6 and R7 (Table 1).

Table 1: Resistance types in Irish *M. graminicola* populations

R type	Mutations/alterations to target site
R5	V136A, Y461S
R6	I381V, Y461H
R7	±A379G, I138V, Δ459/460
R type(new)	V136A, Y461S, S524T

The R5 group is sensitive to the triazoles epoxiconazole, prothioconazole, tebuconazole and metcomazole. R6 and R7, because they carry mutations I381V, have reduced sensitivity to metconazole and tebuconazole (more so to tebuconazole), but they are more sensitive to prochloraz than R5.

Sequencing analysis at Oak Park of the triazole target site in the septoria isolates with the highest levels of insensitivity to epoxiconazole and prothioconazole (EC50>10µg ml^{-1}) detected in 2008 showed that they were similar to the R5 isolates previously detected but with an additional mutation S524T. This represents a new genetic strain of *M. graminicola* not detected in Ireland before. In addition to reduced sensitivity to epoxiconazole and prothioconazole these new strains also has reduced sensitivity to prochloraz similar to the R5 isolates. They do not have mutation I381V so they remain sensitive to metconazole and tebuconazole.

Leroux, P, Albertini C, Gautier A, Gredt M and Walker A S (2007): Mutations in the CYP51 gene correlated with changes in the sensitivity to sterol 14a-demethylase inhibitors in field isolates of *Mycosphaerella graminicola*. *Pest Management Science* 63, 688-698.

The effect of minimum tillage on the production of spring barley and oilseed rape and an assessment of it's impact on soil characteristics and soil fauna.

Forristal, P.D., Kennedy, T., Murphy, K. and Connery J **RMIS No. 5615**

This project which commenced in 2007 has the following key objectives

- To determine the effects of minimum tillage on components of yield, grain yield, grain quality and the profitability of spring barley and winter wheat production.
- To assess the effects of cultivation system on crop development and seed yield in oilseed rape production systems.
- To study the stratification of nutrients and soil organic matter in soils subjected to alternative cultivation strategies for more than 6 years.
- To study the interaction between cultivation system and Nitrogen response in a spring barley crop.
- To complete the investigations of the effect of cultivation system type on aphids and virus infection of winter cereals.
- To determine the risk of plant density reduction due to slug damage on min-tilled wheat and to assess the effect of tillage system on aphid numbers in wheat ears.
- To assess the effects of cultivation system on: a) earthworm populations and species composition; and b) on the species diversity and abundance of the pest-predator family of ground beetles.

Winter wheat

The Knockbeg site was subjected to similar cultivation treatments for 9 years prior to the reported season. The basic experiment is laid down as a randomised block with 4 replications. The cultivation and straw incorporation treatments are:

- Conventional plough-based system with straw baled and removed.
- Conventional plough-based system with straw chopped and incorporated.
- Reduced cultivation (Min-Till) with straw baled and removed.
- Reduced cultivation with straw chopped and incorporated.

Since 2008 within each of these cultivation plots, there are 10 sub-plots with a 5 x 2 arrangement of applied nitrogen rates and seed rates respectively.

The conventional cultivation (PL) consists of ploughing to 200-250 mm, followed by a single pass of a rotary power harrow to 100-120 mm. Reduced cultivation (MT) consists of one pass in August/September with a 3-metre wide tine cultivator (Horsch), working about 70-80 mm deep, followed immediately by a consolidating run with a roller. This is followed by an interval of about 3 weeks to allow weeds and volunteer cereals to become established, prior to herbicide application. All crops are sown with a cultivator drill (Vaderstad Rapid) modified to produce 2.7m wide plots. Two seed rates are used to increase the probability of getting similar plant populations with the two establishment systems. The nominal seed rate settings for this season were 300 and 350seeds per m^2. All plots were sown on the 13th October in fair soil conditions. Weeds were effectively controlled with a conventional autumn programme using pendimethalin and isoproturon for grass and broad leaved weeds, followed by a spring application of sulfosulfuron to control sterile brome. A conventional three spray fungicide programme was used for disease control. Five applied N rates: 0, 160, 200, 240 and 280 kg N per hectare were applied in a three split programme. Growing conditions were not good for the 2009 harvest year. Overall yields were low in 2009 (yield at 200kg N/ha given in table 1). Considerable levels of take-all were visible across the plots. There was no significant difference in grain yield between the main treatments although there was a tendancy for the incorporation of straw to reduce subsequent grain yield (P=0.09). Grain quality was good with cultivation or straw incorporation having no effect on the parameters measured.

Table 1: Plant counts, grain yield and quality, winter wheat, Knockbeg, 2008-2009

Treatment	Plant count (n)	Yield @ 85% DM (t/ha)	Hectolitre weight (kg/hl)	Screenings < 2 mm (%)
PL - straw		8.24	72.1	2.3
PL + straw		8.15	71.9	2.9
RC - straw		8.59	72.5	2.8
RC+ straw		7.89	72.2	2.9
Sig		*ns*	*ns*	*ns*

Preliminary analysis of the response to applied nitrogen indicates a similar response function across both establishment systems with and without straw incorporation but with a tendency for the min-till without straw incorporation to have the highest yield at most of the applied N rates (Fig 1). The year did seem to favour the use of higher N rates with little evidence of the yield plateau at the highest applied N rates for this year.

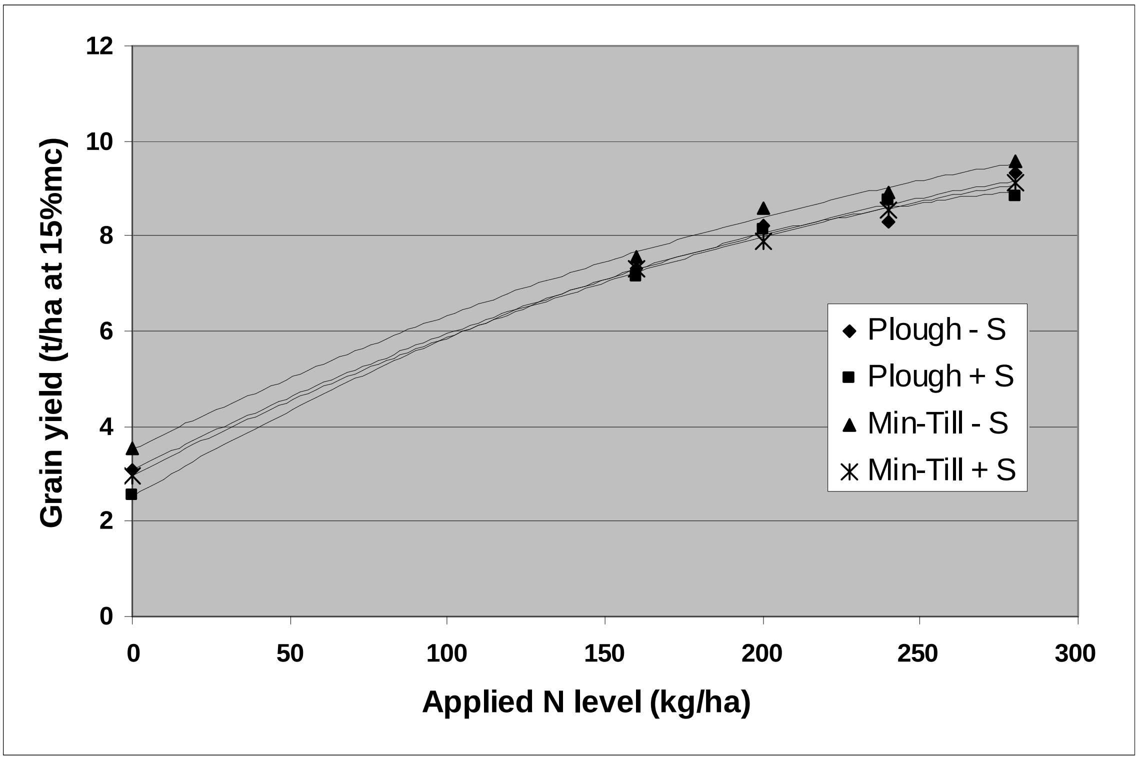


Fig 1: Nitrogen response curves for plough and min-till established winter wheat

Spring Barley – Knockbeg

A replicated field trial evaluating cultivation systems, applied nitrogen fertiliser rates, and seed rates was set-up in 2007. The trial site is on a medium to heavy textured soil located at Knockbeg, which presents a greater challenge to the cultivation systems than the more easily worked soils at Oak Park where the previous spring barley observations were located. The main cultivation treatments are:

- Conventional establishment: Ploughing followed by secondary cultivation with a power harrow prior to sowing.
- Min-till Autumn: Stubble cultivation in the autumn followed by sowing in the spring
- Min-till Autumn and Spring: Stubble cultivation in the autumn followed by a second similar cultivation in spring prior to sowing.
- Min-till Spring: Stubble cultivation in the spring only followed by sowing

Within each of these plots, two seed rates (300 and 350 seeds/m^2) and five Nitrogen rates (0, 75, 105, 135, 165 kg/ha) were applied in a 5 x 2 factorial arrangement. For the 2009 harvest season, the treatments that were stubble-cultivated in autumn were tilled on Sept 24th. All treatments were sprayed with glyphosate on Feb 13th with the appropriate plots ploughed on Feb 25th. Secondary cultivations, where appropriate, were applied on March 23rd with all plots sown on March 24th. Soil conditions were visibly satisfactory at the time of sowing. The Nitrogen was applied on a two-split basis and all plots received a herbicide programme which included grass weed control, and a standard two-spray fungicide programme. Measurements included: plant establishment; components of yield and grain yield and quality.

The effect of cultivation system and nitrogen rate on crop yield is summarised in Fig 2. Plot yields were only moderate on this site. In contrast to the previous year, with the Min-till treatments, autumn cultivation alone tended to give the highest yields with spring cultivation on its own having the lowest yield. As with previous years however, plough based establishment gave yields at least as good as all other min-till treatments, resulting in the plough establishment system being more reliable on this site.

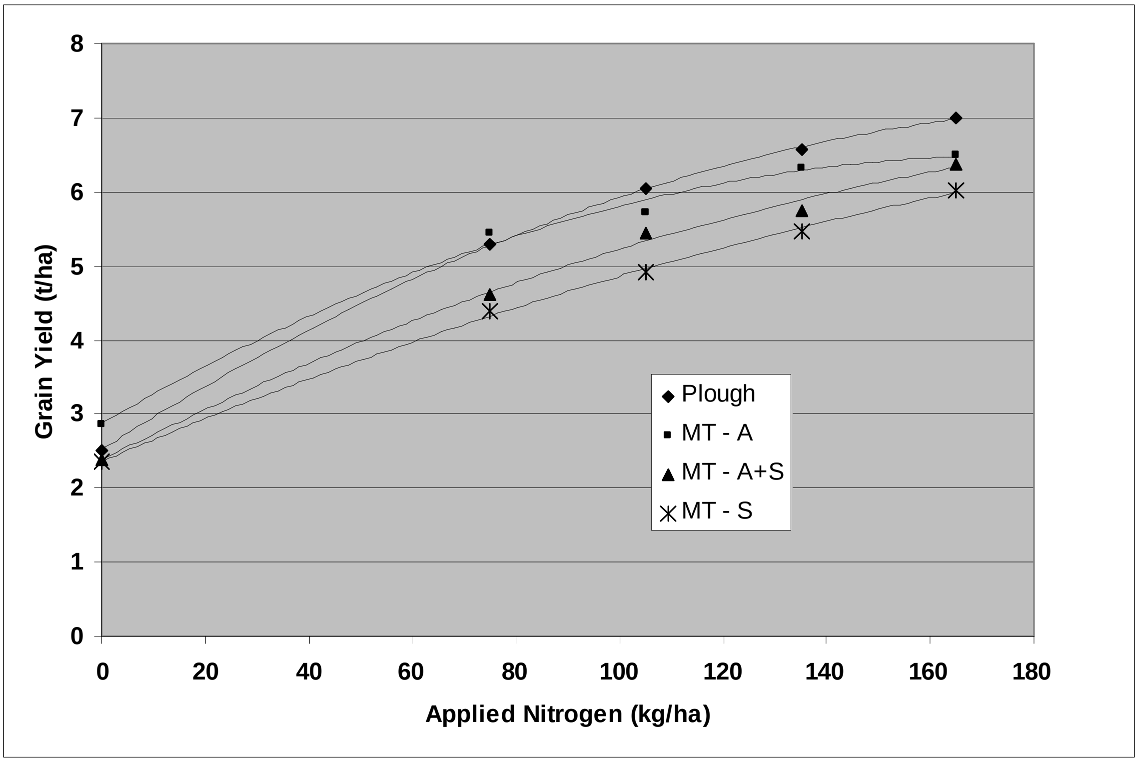


Fig 2: The effect of cultivation system and N rate on spring barley yield (350seed/m2). 2009

Winter barley

The performance of winter barley under PL and MT cultivation systems is assessed in a non-replicated observation trial. The systems are evaluated in a 4-hectare field, half of which is ploughed and half established with reduced cultivation. The site is on a free draining gravely sandy loam soil at Oak Park. In this experiment the straw is baled and removed from both treatments. The crop was sown in good soil conditions on 26th September. Establishment levels were similar across both treatments. Yields were very good, with the plough-established crop yielding better than that established by min-till (table 2). Each yield figure in table 2 comprises of 30 yield assessments in each side of the split field. Grain quality parameters were similar with both systems.

Table 2: Plant establishment and grain yield and quality, winter barley, Oakpark (House Field), 2008-2009.

Treatment	Yield @ 85% d.m. (t/ha)	Moisture content (%)	Hectolitre weight (kg/ha)	Screenings <2.2mm (%)
PL	9.65	19.6	66.5	0.5
MT	9.20	18.7	67.5	0.7

Two sequential stale seedbeds are prepared annually post-harvest on the min-till treated section of the field to reduce the weed burden; particularly sterile brome. There was little difference in observed weed control between the two systems in 2008/2009 however there are low levels of sterile brome evident particularly on the min-tilled area of the field.

Spring barley – Oak Park

This non-replicated experiment compares PL and MT cultivation systems for spring barley.

The treatments are as follows.

- PL - straw incorporation
- PL + straw incorporation
- MT - straw incorporation
- MT + straw incorporation

This was the ninth year of the trial and the field had been in continuous spring barley for at least 30 years before this work commenced. The 2-hectare site was divided into two large plots, PL and MT. These areas were further sub-divided into two 0.5-hectare areas on which the straw was baled and removed or chopped and incorporated. Cultivation treatments were similar to the previous year. The crop was sown on March 16th in good soil conditions. Yields were good with the plough based system yielding approximately 0.6t/ha more than the crop established with min-till (Table 3).

Table 3: Grain yield and quality, spring barley,Oak Park (Clonaherk), 2009

Treatment	Yield @ 85% d.m. (t/ha)	Moisture content (%)	Hectolitre weight (kg/hl)	Screenings < 2.2 mm (%)
PL-straw	7.63	17.1	63.2	2.1
PL+straw	7.46	17.4	64.3	1.8
RC- straw	7.00	16.6	64.1	2.2
RC+ straw	6.90	16.5	64.0	2.6

Establishment systems for Winter Oilseed Rape

A trial evaluating alternative crop establishment systems was completed in 2009. The objective was to determine the impact of crop cultivation system, specifically minimum tillage systems on crop yield and components of yield. The cultivation and establishment treatments used were:

- Plough, press/roll, power harrow/drill, roll
- Plough, press/roll, vaderstad drill, roll
- Min-till 75-100mm 1 run, roll, broadcast sow, roll
- Min-till 75 – 100mm 1 run, roll, vaderstad drill, roll
- Mintill 75 – 100mm 2 runs, roll, vaderstad drill, roll
- Min-till 75- 100mm 2 runs, roll, broadcast sow, roll
- Min-till 150 – 200mm 2 runs, vaderstad drill, roll
- Broadcast sow, roll (30% extra seed rate)

Two seeding rates: 80 seeds/m^2 and 54 seeds/m^2 were used with each cultivation treatment in a split plot design. (Note: Treatment H had 30% extra seed sown in both of the two nominal seeding rates to compensate for expected poor establishment) The site chosen for the 2009 harvest year was a moderately heavily textured soil that had a first-wheat crop grown in the previous season. Autumn 2008 weather conditions prevented sowing until September 17th resulting in a more poorly established crop than the previous years with more weed competition, significant bird damage and a poorly developed crop entering the spring of 2009. The cultivation plots were 6m wide x 30m long with 5 replications in a randomised block design. The crop establishment and yield results of all three years of the trial are presented in table 5. Crop establishment varied with the cultivation treatment and year. The conventional plough-based system (A) normally gave good establishment although treatment B was less consistent due to a tendency for the drill working directly on ploughed soil to sow the seeds too deeply. All of the minimum tillage systems (excluding treatment H) proved capable of producing similar yields to plough based establishment. In 2009, these min-till treatment yields tended to be greater than the plough-established crops. The broadcast sown treatment (H) resulted in a significantly poorer yield in 2007 and 2009. Overall most of the min-till systems evaluated allowed successful establishment and good crop yields to be achieved. The broadcast method was less reliable however

Table 5: The effect of cultivation system on winter oilseed rape yields. 2007, 2008, 2009.

Cultivation Treatment	Plant Count (n/m2)			Yield (t/ha 9% mc)		
	2007	**2008**	**2009**	**2007**	**2008**	**2009**
A	104	79	70	5.3	4.7	3.5
B	53	56	54	5.3	4.6	3.3
C	89	55	61	5.5	4.8	4.5
D	80	56	60	5.3	4.7	4.4
E	79	59	61	5.6	4.8	4.2
F	75	56	68	5.4	5	4.6
G	69	57	65	5.5	4.7	4.2
H	76	26	51	5.1	4.6	3.0
Sig	***	***	***	**	ns	***
s.e.d.	*9.0*	*4.3*	*2.8*	*0.11*	*0.15*	*0.27*

Crop production systems: a study of key factors in their performance; long-term effects of rotation and input level; and effective validation and dissemination

Forristal P.D, Burke. B **RMIS No: 5616**

The overall objectives of this project are:

- To determine the interaction between seed rate, applied nitrogen rate and fungicide level in winter wheat.
- To continue studies on the effect of system including input level and rotation type on crop yields and production margin on high and moderate disease pressure sites.
- To compare the effects of 10+ years of monoculture and rotation and input level on soil characteristics, weed flora and soil fauna.

Methods

The trial is primarily carried out at the Knockbeg site adjacent to Carlow with some supporting research carried out at Kildalton. Rotations and input levels are being evaluated at Knockbeg. The input strategies evaluated at Knockbeg include:

- **High:** Commercial levels of all fertiliser, herbicide, fungicide and other inputs. This is a high-yield, low-risk strategy which is easily managed, but tends towards high production costs.
- **Low:** Nitrogen levels reduced to approximately 80% of the 'High' strategy with all other chemical inputs reduced to 50% of the 'High' rate. This is a reduced yield, moderate risk, but easily managed strategy with lower input costs.
- **Decision-based(DB) High:** Input levels are in-part determined by a decision making process aimed at optimising response to the input. This is a high-yield, moderate risk strategy requiring greater management levels than the fixed input levels outlined above. (winter wheat and spring barley only)
- **Decision-based(DB) Low*:*** Low levels of inputs which are in-part determined by a decision making process aimed at minimising costs while maintaining adequate yield. This is a reduced yield, high-risk strategy. (winter wheat and spring barley only)

Two rotations: a five course break-crop rotation incorporating a legume and oilseed crop with three cereals; and a three course all-cereal rotation with oats preceding wheat, are grown in comparison with continuous winter wheat and spring barley crops at the Knockbeg site. The basic plot (12.5m x 30m) constitutes an individual crop grown with either high or low levels of inputs with decision-based inputs used on one half of these plots in a split-plot design. Each individual treatment is replicated four times. Decision based strategies are based on a combination of advisory decisions using crop and weather knowledge and computer-based decision support systems (DSS). The DSS systems, which were only used for winter wheat and spring barley (Knockbeg) disease control, were the Danish 'Pro-plant' system for the 'DB-High' strategy on both cereals and the 'DB-Low' strategy on spring barley. The less complex 'Septoria Timer' system was used for the wheat 'DB-Low' approach. As the Danish DSS system recommends extremely low rates of fungicide, these rates were doubled on the DB-high strategies.

Results: Growing conditions in 2008 / 2009 were not generally favourable for cereals on the Knockbeg site with the exception of winter barley which performed well. Wet weather dominated the growing season impacting on soil conditions, nitrogen and disease levels.
W. Wheat and Input levels: Wheat yields were moderate to poor compared with previous years on this site (Table 1). The use of high input levels gave a 1.1t/ha or 13% yield advantage over the reduced inputs. This yield advantage would not however cover the extra costs associated with the 'high' input strategy where, depending on input and grain prices, an extra yield of 1.4 t/ha is typically needed to cover the extra production costs. This continues the trend of previous years where the low input strategy is generally more profitable with the exception of years with very high yield potential and/or high grain prices. The 'decision-based' strategies which used the modified output of a Danish decision support system to determine the timing and products used for disease control, allowed broadly similar yields to be achieved with a small saving in fungicide costs. Protein levels were low across all treatments; in particular the values recorded with the low input levels indicated that the applied nitrogen levels were influencing yields.

S. Barley and Input levels*:* Spring barley yields for the 'high' input system were the lowest ever achieved on this site since the trial began (Fig 1). Unlike other years, the yield difference between 'high' and 'low' input levels was very small at less than 5%, which resulted in the low input being most profitable. This result differs from previous years where the high input approach normally gave about 20% higher grain yield than the low input strategy. The Knockbeg site can be difficult for spring barley and 2009 was a very difficult year. The crop was sown relatively early (Mar 16th), when ground conditions were marginal. While the crop established satisfactorily, foliar growth was poor with the crop displaying nitrogen deficiency symptoms through most of the growing season. It's likely that root development was restricted by a combination of marginal soil conditions at sowing combined with wet weather immediately post sowing. This, coupled with the summer rainfall of 2009 being almost twice the average amount, resulted in plants with poor nitrogen uptake and consequently poor growth and yield (Table 1). The protein levels recorded indicate significant nitrogen deficiency. The DSS system used to determine the fungicide programme in the decision-based strategies had little impact on yield.

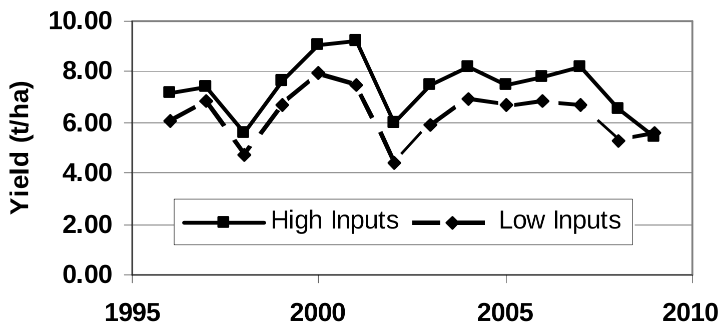


Fig 1: Spring barley yield trends 1996 to 2009 for high and low input levels

Winter Barley and Winter Oats: In 2009 the winter barley crop yielded reasonably well with quite good yields achieved with high input levels. The low-input approach reduced yield by approximately 1.0 t/ha which resulted in the financial margin from the two systems being approximately equal. The yield difference between high and low input systems in winter oats was similar at approximately 1.0t / ha resulting in the high input strategy being more profitable with this crop in 2009.

Table 1: Effect of input strategy on grain yield, Knockbeg 2009

Cereal crop	Input strategy	Yield (t/ha)	Specific Gravity (kg/hl)	Protein (%)
Winter wheat	High	9.40	71.9	9.4
	DB-High	9.33	71.3	9.6
	Low	8.30	70.8	8.0
	DB-Low	8.50	70.8	8.1
Winter barley	High	9.00	67.3	11.6
	Low	7.88	67.1	10.7
Winter oats	High	7.92	55.8	
	Low	6.79	56.7	
Spring barley	High	5.14	63.1	7.7
	DB-High	4.84	62.0	7.8
	Low	4.90	61.8	7.6
	DB-Low	4.94	61.5	7.4

Rotation effect*:* The effect of rotation on cereal crop performance was evident in all cereals in the trial where this effect could be monitored. Consistent with previous years results, the wheat grown after oats gave the highest yield (1.2 t/ha more than monoculture) followed by wheat after beans; with continuously cropped wheat having the lowest yield (Table 2). The preceding bean crop may not be supplying sufficient available N to the following wheat crop to compensate for the 50kg N/ha reduction in the wheat N allowance. With winter barley, where the crop was grown after spring oilseed rape, it produced about 1.1t/ha more grain than when the crop followed a wheat crop in the cereal rotation. When monoculture spring barley was compared with spring barley grown as the second crop after beans in a break crop rotation, there was a 1.1t/ha yield benefit to the barley grown in the rotation. This yield difference is proportionately larger than previous years. It may be that the apparent poor utilisation of applied nitrogen to the spring barley crop in 2009 (discussed above) allowed a soil nitrogen benefit from the bean crop grown two seasons previously to be expressed.

Table 2: The effect of rotation on cereal yields: Knockbeg 2009

Crop	Rotation and previous crop	Yield (t/ha)	Protein (%)	Specific Wt (kg/hl)
Winter wheat	Break crop; Beans	9.00	9.0	71.5
Winter wheat	Cereal Rotation; Oats	9.41	8.9	71.7
Winter wheat	Monoculture	8.20	8.5	70.4
Winter Barley	Break Crop; Rape	9.00	11.0	66.8
Winter Barley	Cereals rotation; W.Wheat	7.88	11.1	67.7
Spring Barley	Break Crop; W.Wheat	5.51	7.7	61.7
Spring Barley	Monoculture	4.45	7.6	62.5

The evaluation of new atrazine-replacing herbicides for their effectiveness in controlling broadleaved and grass weeds in forage maize crops

Hackett, R. **RMIS No. 5618**

Trials examining weed control in maize crops sown with and without a plastic mulch were continued in 2009 at two sites, Knockbeg and Kildalton. Details of sites and treatment applications are given in Tables 1 and 2.

Weed control in crops grown with a plastic mulch

The main weeds present in the crop sown under plastic at Knockbeg were redshank, nightshade, fools parsley, amg, fumitory, bindweed and OSR. The majority of treatments gave good control of weeds under the plastic (Table 3). The products giving less than satisfactory control under plastic were Calaris, Cadou Star and Templar used without a tank-mix partner. Calaris and Templar alone gave poor control of nightshade under the plastic while Cadou Star alone gave poor control of redshank and bindweed under the plastic.

Weed control in interrow spaces without plastic cover was much more variable. Generally grassweeds were present at only low frequency so only BLW were assessed. The best control was given by a mixture of Calaris and Cadou Star, while satisfactory levels of control were given by most mixtures containing Pendimethalin (PDM) and either Calaris or Cadou Star. Pendimethalin applied alone gave poor weed control in the areas not covered with plastic. Redshank and fools parsley were the main weeds present in the pendimethalin only treatments.

At Kildalton the main weeds present were fat hen, orache, nightshade, annual meadow grass and charlock. Very good control of weeds in the plastic uncovered areas was given by pendimethalin in mixture with either Calaris, Cadou Star or Templar. Calaris applied alone also gave good control of weeds between the rows of plastic. Both Calaris and Templar gave poor control of nightshade under the plastic. The remaining treatments all gave good control under the plastic.

Weed control in crops grown without a plastic mulch

The main broadleaved weed species present in the Knockbeg trial were redshank, nightshade, bindweed, fools parsley and fumitory. A range of other species including oilseed rape, fat hen, sun spurge, shepherds purse and woundwort were present at lower frequency. Overall very good control was achieved in all treatments that received Calaris either alone or in combination with PDM (Table 4). Laudis and Templar treatments also gave very good control. The mixture of Callisto and Samson Extra and the treatment that received BAS659 pre-emergence followed by Callisto post emergence also gave very good control. A number of treatments gave poor control including Pendimethalin alone or in combination with Grounded, Cadou Star applied alone, and Titus applied with Agral. Cadou Star applied alone gave poor control of bindweed which was the principal weed remaining and only moderate control of redshank. Pendimethalin applied alone pre-emergence gave poor control of redshank and fools parsley and moderate to good control of nightshade, particularly when combined with Grounded. Applied alone post emergence it gave poor control of redshank and only moderate control of bindweed. Mixing Cadou Star and Pendimethalin improved control of bindweed to some extent but did little for redshank. Laudis appeared to give lower levels of control of fumitory than some other treatments.

Grassweed levels were low in the Knockbeg trial so it was difficult to accurately assess the effectiveness of the products. The main species present was AMG and it was adequately controlled by the majority of the treatments with the post-emergence application of pendimathalin and the split Bromotril treatment and Laudis treatments giving less control than the remaining treatments.

Weed population densities were much lower at Kildalton than at Knockbeg. Charlock was the predominant weed in the trial. Orache and fat hen were also present at moderate to high levels while speedwell and knotgrass occurred at moderate levels. At Kildalton Cadou Star treatments gave much better levels of control than at Knockbeg (Table 4); neither redshank nor bindweed was prevalent at Kildalton. In general treatments which performed well in Oak Park also performed well at Kildalton, where included.

Both the application of the first and second (where applicable) post emergence applications were delayed somewhat due to poor conditions which may have disadvantaged these treatments. The lower level of broadleaved weed control in the split Templar treatment compared to the single Templar treatment was most likely due to this delay. The reduction in control achieved with the Calaris (1 l/ha) treatment relative to Oak Park appeared to be due to reduced levels of control of orache and to a lesser extent groundsel. The reduced level of control achieved with the Callisto + Samson Extra treatment at Kildalton was largely due to poor control of speedwell.

Grass weeds were more prevalent at Kildalton than at Knockbeg, the main species' present being annual meadow grass and volunteer oats. The oats in particular was not evenly distributed across the trial making comparisons between treatments difficult. The Bromotril split treatment and pendimethalin applied alone post emergence gave the poorest level of control largely as a result of poor control of annual meadow grass, but also fat hen and orache in the case of the Bromotril treatment.

Table 1: Details of weed control trials in maize grown with a plastic mulch at Knockbeg and Kildalton on 2009

	Knockbeg	Kildalton
Sowing date	May 6	May 7
Variety	Justina	Justina
Plastic type	'Xtend'	'Samco black'
1st assessment	June 15	June 29
2nd assessment	July 15	July 29

Table 2: Details of weed control trials in maize grown without a plastic mulch at Knockbeg and Kildalton on 2009

	Knockbeg	Kildalton
Sowing date	May 12	May 7
Variety	Justina	Loft
Pre-emergence herbicides	May 15	May 12
1st post emergence herbicides	June 11	June 11
2nd post emergence herbicides	July 3	July 8
1st assessment	June 15	June 29
2nd assessment	July 2	July 8
3rd assessment	July 20	July 29

Table 3: Effect of herbicide programme on weed control (% control relative to untreated) in maize grown using a plastic mulch at Knockbeg and Kildalton in 2009

	Knockbeg				Kildalton					
	June 15		July 15		June 29		July 29			
Treatment	BLW Under	BLW Between	BLW Under	BLW Between	BLW Under	BLW Between	BLW Under	BLW Between	GRASS under	GRASS between
No herbicide										
Stomp Aqua (4 l/ha)*					100 a	95 cd	100 a	88 d	77 c	90 bc
Stomp Aqua (5 l/ha)*	99 a	71 defgh	99 ab	65 defgh						
Cadou Star (0.75 kg/ha)	38 c	58 hi	38 f	50 fghi						
Calaris (1.5 l/ha)	46 c	81 abcde	42 f	72 bcde	81 c	97 bc	75 b	90 cd	100 a	96 ab
Calaris (1.5 l/ha) + Cadou Star (0.75 kg/ha)	99 a	94 a	98 abc	95 a						
PDM (2.4 l/ha) + Calaris (0.75 l/ha)	99 a	88 abc	100 a	81 abcd						
PDM (2.4 l/ha) + Calaris (1.5 l/ha) + Grounded (0.4l/ha)	99 a	92 a	100 a	85 abc						
PDM (4.5 l/ha)	90 a	46 i	92 bcdef	29 i	96 b	85 ef	98 a	73 e	81 c	85 c
PDM (4.5 l/ha) + Bromotril (0.9 l/ha)	93 a	46 i	90 cdef	29 hi						
PDM (4.5 l/ha) + Cadou Star (0.5 kg/ha)	99 a	90 ab	92 bcde	81 abcd						
PDM (4.5 l/ha) + Cadou Star (0.56 kg/ha)					100 a	99 a	100 a	93 bc	99 ab	100 a
PDM (4.5 l/ha) + Cadou Star (0.75 kg/ha)	97 a	91 ab	97 abcd	81 abcd						
PDM (4.5 l/ha) + Calaris (0.75 l/ha)	100 a	91 ab	99 ab	91 ab						
PDM (4.5 l/ha) + Calaris (1 l/ha)	98 a	85 abc	100 a	75 bcde	100 a	100 a	100 a	99 a	100 a	100 a
PDM (4.5 l/ha) + Callisto (1.25 l/ha)	97 a	77 bcdef	94 abcd	71 bcdef						
PDM (4.5 l/ha) + Grounded (0.4 l/ha)	96 a	63 gh	92 bcde	46 ghi						
PDM (4.5 l/ha) + Templar (1.1 l/ha)	100 a	69 efgh	92 bcde	54 efghi						
PDM (4.5 l/ha) + Templar (2.5 l/ha)	100 a	75 cdefg	100 a	65 cdefg	100 a	98 ab	100 a	96 ab	100 a	100 a
PDM (4.5 l/ha) + Templar (2.5 l/ha) + Grounded (0.5 l/ha)	100 a	83 abcd	100 a	65 defg						
PDM (6 l/ha)	100 a	58 hi	90 def	54 efghi						
PDM 2.4 l/ha + Calaris 1.5 l/ha	100 a	93 a	100 a	83 abc						
Templar (2.5 l/ha)	65 B	67 Fgh	54 ef	33 i	29 c	71 f	29 b	50 e	100 a	100 a

maximum label dose is 3.3 l/ha

PDM = Alpha Pendimethalin 330 EC

Table 4 Effect of herbicide programme on weed control (% control relative to untreated) in maize grown without plastic at Knockbeg and Kildalton in 2009

		Knockbeg					Kildalton					
		June 15	July 2		July 20		June 29		July 8		July 29	
Treatment		Overall	Grass	BLW	Grass	BLW	BLW	Grass	BLW	Grass	BLW	Grass
No herbicide												
Stomp Aqua* (4 l/ha)	Pre	85 bcd	99 a	73 ef	100 a	58 efg	85 a	79 bc	89 ab	77 a	83 abcd	79 bc
Stomp Aqua* (4 l/ha)							88 a	94 ab	98 a	95 a	92 abc	95 ab
Bromotril (1.5 l/ha) + Bromotril (1 l/ha)[1]	Post		77 d	83 bcde	73 c	85 abc	56 bc	60 d	67 c	54 b	79 bcd	58 e
Cadou Star (0.75 kg/ha)	Pre	77 d	100 a	73 ef	99 a	56 efg						
Cadou Star (0.85 kg/ha)	Pre						88 a	96 ab	83 ab	96 a	79 bcd	92 ab
Calaris (1 l/ha)	Post		100 a	99 a	100 a	99 a	97 a	84 abc	98 a	81 a	88 abcd	88 abc
Calaris (1.5 l/ha)	Post		100 a	99 a	100 a	100 a	100 a	92 abc	98 a	92 a	92 abc	92 ab
Callisto 1.0 l/ha + Samson Extra 0.5 l/ha	Post		99 a	97 a	100 a	95 ab	92 a	100 a	89 ab	100 a	79 bcd	97 a
Laudis (1.7 l/ha)	Post		90 b	95 ab	92 ab	93 ab						
Laudis (2.25 l/ha)	Post		89 bc	94 abc	81 bc	94 ab						
PDM (2.4 l/ha) + Calaris (0.75 l/ha)	Post		100 a	98 a	96 ab	100 a	98 a	90 abc	98 a	87 a	94 ab	87 abc
PDM (4.5 l/ha)	Pre	77 d	96 ab	67 f	91 ab	50 fg	94 a	75 cd	92 a	77 a	88 abcd	73 cd
PDM (4.5 l/ha) + Cadou Star (0.5 kg/ha)	Pre	94 ab	100 a	81 cde	100 a	67 def	100 a	89 abc	100 a	91 a	98 a	94 ab
PDM (4.5 l/ha) + Cadou Star (0.75 kg/ha)	Pre	99 a	100 a	89 abcd	100 a	79 bcd	100 a	97 a	100 a	96 a	100 a	97 a
PDM (4.5 l/ha) + Calaris (0.75 l/ha)	Post		99 a	98 a	100 a	94 ab	98 a	90 abc	100 a	88 a	93 ab	88 abc
PDM (4.5 l/ha) + Calaris (1 l/ha)	Post		100 a	99 a	100 a	99 a	98 a	92 abc	100 a	96 a	100 a	92 ab
PDM (4.5 l/ha) + Grounded (0.4 l/ha)	Pre	79 cd	96 ab	73 ef	85 abc	56 efg	81 a	85 abc	85 ab	79 a	81 abcd	79 bc
PDM (4.5 l/ha) applied post emergence	Post		81 cd	48 g	88 abc	42 gh	50 c	63 d	65 c	54 b	73 cd	60 de
PDM (6 l/ha)	Pre	88 bc	92 ab	79 def	92 ab	69 cde						
Templar (1.5 l/ha) + Templar (1.0 l/ha)[1]	Post		96 ab	91 abcd	100 a	91 ab	75 ab	83 abc	73 bc	88 a	71 d	92 ab
Templar (2.5 l/ha)	Post		100 a	98 a	94 ab	92 ab	98 a	88 abc	94 a	87 a	90 abcd	88 abc
Titus (40 g/ha) + Bromotril (0.75 l/ha)	Post		100 a	83 bcde	98 a	73 cde	54 bc	92 abc	33 d	97 a	46 e	94 ab
Titus (50 g/ha) + Agral (0.1%)	Post		100 a	23 h	100 a	29 h	42 c	96 ab	38 d	100 a	50 e	98 a

* maximum label dose is 3.3 l/ha PDM = Alpha Pendimethalin 330 EC

A comparison of the effect of cultivation system and overwinter cover on spring barley grain yield and nitrate concentrations in the soil solution over the winter period

Hackett R. **RMIS 5179**

Studies on the effect of overwinter cover on grain yield of spring barley in a plough-based cultivation system and a reduced tillage system were continued at Oak Park in 2009. Three overwinter covers were compared, no vegetative cover, a mustard cover crop and natural regeneration consisting of volunteer barley and weeds.

The natural regeneration and mustard cover crop were sprayed with glyphosate mid-March. Plough treatments were ploughed in early April before sowing. Reduced cultivation treatments were cultivated in autumn 2008 after harvest of the previous cereal and the only cultivation in spring 2009 was with discs of a Vaderstad drill during the sowing operation. All crops received standard pesticide and fertiliser N inputs with the exception of small areas (2m x 2m) within each plot which received no fertiliser N. These areas were used to determine the effects of the various treatments on growth and N uptake of spring barley in the absence of fertiliser N.

Yields were low in all treatments due to poor tillering in the barley as a result of poor growing conditions in May and June. Harvesting losses were also high due to considerable straw breakdown in all treatments. There was no significant effect of overwinter cover on grain yield (Table 1). Grain yield was significantly greater in the plough based system than in the reduced cultivation system but the difference between the two cultivation systems was small (0.3 t/ha). There was not a significant interaction between cultivation system and overwinter cover.

There was no significant effect of either overwinter cover or cultivation method on hectolitre weight.

There were no significant effects of cultivation method on 1000 grain weight. The no cover treatment had a significantly lower 1000 grain weight than both other overwinter treatments.

There was a significant effect of overwinter cover and cultivation method on grain protein content. Grain protein was significantly higher in the reduced cultivation treatment than in the plough treatment. Grain protein did not differ significantly between the mustard and natural regeneration treatments. The no cover treatment gave significantly lower protein content than either mustard or natural regeneration treatments.

Table 1. Effect of cultivation method and overwinter cover type on yield and quality of spring barley (cv. Wicket) at Oak Park in 2009.

Cultivation method	Overwinter cover	Yield (t/ha)	Hectolitreweight (kg/hl)	1000 grain wgt (g)	Protein content (%)
	Natural regeneration	4.1	62.4	45.7	9.1
Plough based	Mustard	4.3	62.7	45.4	9.0
	No cover	3.9	62.4	43.0	8.7
	Natural regeneration	3.7	62.1	43.7	9.2
Reduced cultivation	Mustard	3.8	63.6	45.4	9.5
	No cover	3.7	62.3	41.7	8.8
Cultivation method (A)		0.19*	ns	ns	0.11*
Overwinter cover (B)		ns	ns	1.59	0.14*
A x B		ns	ns	ns	ns

* 5% LSD ns = non significant

In the absence of fertiliser N barley grain yield was significantly increased where mustard was the overwinter cover when compared to bare soil (Table 2). Grain yield was numerically higher in the natural regeneration treatment compared to eth no cover treatment but the difference was not statistically significant. There was no effect of tillage on grain yield or no interaction between over winter cover and tillage method. There was no significant effect of tillage method or overwinter cover on ear population density, grains per ear or 1000 grain weight.

Table 2. Effect of cultivation method and overwinter cover type on grain yield and components of grain yield of spring barley (cv. Wicket) in the absence of fertiliser N at Oak Park in 2009.

Cultivation method	Overwinter cover	Ears/m^2	Grains/ear	1000 grain wgt (g)	Grain yield (g DM/m^2)
	Natural regeneration	362.0	11.4	44.4	153.8
Plough based	Mustard	349.0	12.7	43.3	163.6
	No cover	369.5	9.2	41.7	119.6
	Natural regeneration	334.0	11.9	42.0	140.9
Reduced cultivation	Mustard	408.7	11.0	43.8	157.7
	No cover	290.5	10.6	42.9	111.3
Cultivation method (A)		ns	ns	ns	ns
Overwinter cover (B)		ns	ns	ns	34.1*
A x B		ns	ns	ns	ns

* 5% LSD ns = non significant

An investigation of the effects of bio-solids on the invertebrate fauna of agricultural soils

Kennedy T, Artuso N, Connery J. **RMIS: 5617**

Laboratory and field investigations on the effects of biosolids (biofert) on the soil and field invertebrates, including Collembola, Annelida and Coleoptera, were continued in 2009. In the laboratory, biosolids from five locations in Ireland and used at rates equivalent to 0, 2, 5, 10 and 20 t/ha were investigated for effects on the annelid worm *Eisenia fetida* (S.) and the Collembolan *Folsomia candida* (W). The results showed that biosolids at 2 t ha^{-1} and 5 t ha^{-1} rates had no effect on the mortality of adult earthworms. However, at the 10 t ha^{-1} rate a significant reduction in worm numbers was recorded for the combined five biosolids with a further significant reduction for the 20 t ha^{-1} rate. The latter rate resulted in complete mortality of worms for two of the five biosolids. Of the three biosolids investigated at the 2 t ha^{-1} rate only one produced significantly fewer juvenile worms when compared with controls. All biosolids had significantly fewer juveniles relative to controls for rates of 5, 10 and 20 t ha^{-1}. Significant differences in earthworm adult mortality, adult mass and juvenile numbers between biosolids were recorded.

The number of adult Collembola surviving individual biosolids, applied at the 2 t ha^{-1} rate, were not significantly fewer than untreated controls. Increasing the rate from 2 t ha^{-1} to 5 and 10 t ha^{-1} did not impact on the number of adult Collembola but at 20 t ha^{-1} there were significantly fewer adults. Reproduction of juvenile Collembola was a more sensitive parameter than adult mortality in determining the deleterious effects of biosolids. Biosolids at each of the four rates investigated had significantly fewer juveniles relative to controls. When compared with the 2 t ha^{-1} rate, the rates of 5, 10 and 20 t ha^{-1} produced significantly fewer juveniles. Juvenile numbers did not differ between 5 and 10 t ha^{-1} but did for comparisons between 5 and 20 t ha^{-1}. Biosolids from five sources differed in the number of juvenile Collembola produced and showed a similar trend to that recorded for worms.

Based on the chemical analysis of the five Irish biosolids in this study, the element determining the maximum application rate, in compliance with regulations, was phosphorous. The maximum rates at which these biosolids could be applied to grassland (for a P index 3 soil) was 1.7 t ha^{-1} and for cereals was 2.2 t ha^{-1}. It is concluded from these laboratory based assays that biosolids, applied at rates as low as 2 t ha^{-1} are not detrimental to earthworms or adult Collembola but are detrimental to juvenile Collembola.

Field trials investigating the effects of biofert and biocake on earthworms and Collembola in grassland and cereal fields were undertaken in the period 2007 to 2009. Worm density in grass and spring barley plots treated with either biofert or biocake, over three seasons, did not differ significantly when compared with untreated control plots. Similar comparisons for Collembola showed there were significantly greater numbers in both biofert and biocake treated plots when compared with untreated plots. In spring barley, Collembolan density did not significantly differ between treated and untreated plots.

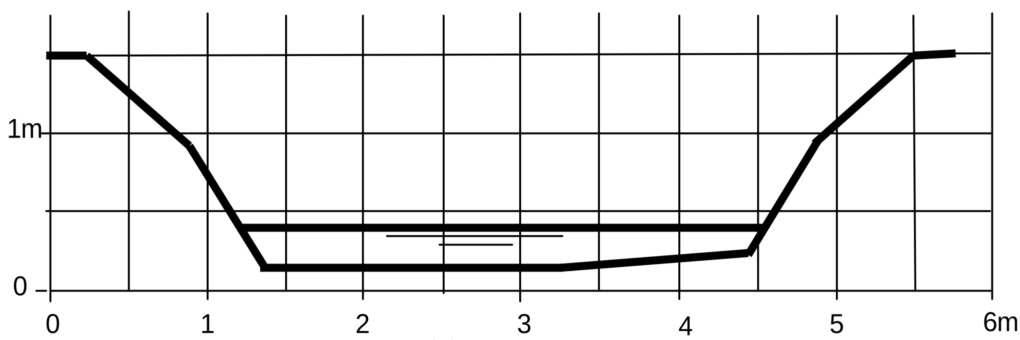
Mini Catchments Project – Procurement and Hydraulic Design

Ryan, D.T. **RMIS No: 5866**

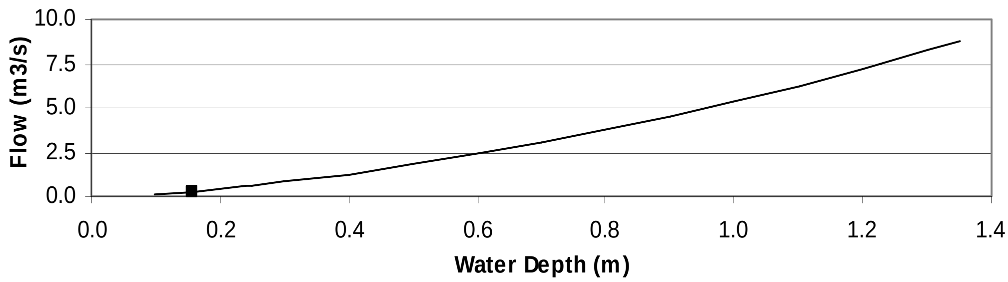
The Agriculture Mini-Catchment Programme is a national, partnership-based project which aims to promote and maintain profitable, productive farming in a clean environment. The programme will be delivered by an integrated team of Teagasc researchers and advisers who work with farmers in a number of intensively farmed mini-catchments where water quality targets are an issue, primarily in relation to the Nitrate Directive. This project is funded by the Department of Agriculture, Food and Fisheries. The initial time frame considered is 2008 - 2012.

Each Catchment includes the headwater of a stream so no influence from outside the catchment boundary was considered to affect the local stream. The location for a base station was identified at a convenient point on each stream where the equipment could continuously record water flow rate, take samples and analyse them there and then. Further sampling equipment was required for use in various locations around the catchment, groundwater equipment was investigated and meteorological stations were planned. The work at Oak Park concerned the collection of this information for the preparation of tender documents which were sent to the project coordinator at Johnstown Castle.

As part of the work, to select equipment for the project, surveys were conducted on two rivers:1. Owenavorragh river near Ballycanew, Bann Catchment, Co. Wexford and 2. The stream at Ballywilliamroe, Castledockrell, Slaney Catchment, Co.Wexford (Fig 1).



(a)



(b)

Fig .1. (a) River cross section at the base station at Castledockrell, Slaney Catchment and (b) a stage curve relating flow to water depth at the cross section. A calibration point is also shown (black dot, Fig 1 (b)).

Survey data were recorded in the vicinity of the base station and 50 to 100 m upstream and downstream of that point to reveal the gradient of the stream bed and bank. The cross-section of the river was measured (Fig 1 (a)) and a float in tube device was installed at this section to record maximum water levels. This information was combined in the standard open channel flow formula by Manning to calculate flow through the channel at any water depth as given in Fig 1 (b). This indicates the flow condition that monitoring equipment is likely to encounter when in place in the streams.

Economic control of diseases of cereals crops and evaluation of the efficacy of fungicide products

Spink, J **RMIS No. 5619**

The appearance of septoria strains with apparently significantly decreased sensitivity to epoxiconazole and prothioconazole in autumn 2008/spring 2009, made the assessment of any impact on field performance a major focus for 2009, (see RMIS 5764 on page 1)

Recent research in the UK has also suggested that early season growth and disease control may be more important in barley, than had previously been thought. It is unknown whether this is also likely to be the case in Irish conditions and work to address this was therefore also prioritised.

Triazole Field Performance against Septoria in wheat.

Since the emergence of septoria with strobilurin resistance and the subsequent breakdown in their field efficacy, winter wheat production in Ireland has been heavily reliant on the triazole fungicides. Septoria tritici blotch caused by the fungal pathogen *Mycosphaerella graminicola* is the primary target of these triazoles and in their absence has the potential to reduce winter wheat yields by anything up to 50%. Even though the triazole fungicides are the largest group of fungicides commercially available, successful septoria control here and throughout most of NW Europe has become dependent on effectively a handful of these chemicals, with epoxiconazole and prothioconazole leading the way.

Reduced performance of the triazole fungicides has developed in a wide range of crops across the world but in most cases complete resistance of the disease is seldom observed. An erosion of sensitivity of the fungus to the fungicide following continued intensive usage, which culminates in reduced efficacy is more common. Triazoles, in one form or other have been applied to wheat since their first invention over 30 years ago. Although little differences in triazole efficacies against septoria were recorded at the full recommended dose over most of this period reductions in their efficacy at reduced doses were observed. Since 2004 / 2005 more significant changes have occurred in the Irish septoria population and populations elsewhere in NW Europe that have altered how we view sensitivity to the triazoles and hence how we use them.

The emergence and dominance of strains of septoria with alterations in their gene coding for the triazole target site, 14α-demethylase, and most notably the mutations V136A and I381V, throughout the 2000s marked a new era for septoria disease control. Although these mutations had little or no effect on the sensitivity of septoria to either epoxiconazole or prothioconazole they did have a significant affect on the sensitivity of septoria to prochloraz and tebuconazole, resepectively, with the I381V also having to a lesser extent an affect on metconazole. The triazole fungicides can no longer be viewed as a single group with the presumption that changes in sensitivity to one will be mirrored by changes in another. Furthermore sensitivity monitoring of septoria from commercial crops and trials carried out by Oak Park demonstrated that selection for these strains was rapid and occurred even after a single application.

The detection of strains of septoria with reduced sensitivity to both epoxiconazole and prothioconazole in the summer of 2008 and their carry over to the spring of 2009 (outlined by Eugene O'Sullivan) was therefore of major concern to Teagasc. These changes in the pathogens population came as a surprise. Both epoxiconazole and prothioconazole had shown an extremely stable and sensitive population for a number of years. Further analysis of these strains at Oak Park identified, both using conventional and molecular techniques that while these strains showed reduced sensitivity to epoxiconazole and prothioconazole they lacked the I381V mutation and were therefore sensitive to both tebuconazole and metoconazole.

On the back of these findings precautionary recommendations were issued. Aware of the importance of epoxiconazole and prothioconazole in septoria control it was concluded that to protect their future activity their usage outside of the application timings most important for septoria control, T1 and T2, should be avoided. Furthermore their use at these timings should always be in mixture with a fungicide with an alternative mode of action against septoria. While such measures were viewed as harsh, the recommendations were issued as an anti-resistance strategy aimed at both minimising selection for these strains while ensuring wheat crops remained protected against septoria. A key part of the anti-resistant strategy the preferred active for use at T3 was metconazole, as its field performance has remained unaffected by the I381V mutation and it was expected to be effective against these new strains which may have been selected by the use of epoxiconazole or prothioconazole at the previous timings.

Whilst lab results can give an indication of what might happen in the field, shifts in sensitivity can never be related directly to effects on field performance. Therefore, to ascertain if the emergence of these strains with reduced sensitivity was going to affect the field performance of the triazole fungicides, programmes trials (Table 1) including straight triazoles, triazoles in mixtures of different actives and sequences of different triazoles were tested at two sites contrasting in the levels of the new strain of septoria present. At the Co. Meath site the strain was present at a high frequency (60%) and at the Knockbeg sites a much lower frequency (5%).

Table 1 Fungicide programmes tested in Meath and Knockbeg

T 1	T 1.5	T 2	T 3
Proline (prothioconazole) (0.8 l/ha)		Proline (0.8 l/ha)	Proline (0.8 l/ha)
Opus (epoxiconazole) (1.0 l/ha)		Opus (1.0 l/ha)	Opus (1.0 l/ha)
Folicur (tebuconazole) (1.0 l/ha)		Folicur (1.0 l/ha)	Folicur (1.0 l/ha)
Bravo (chlorothalonil) (1.0 l/ha)		Bravo (1.0 l/ha)	Bravo (1.0 l/ha)
Bravo (1.0 l/ha)	Bravo (1.0 l/ha)	Bravo (1.0 l/ha)	Bravo (1.0 l/ha)
Inca (epoxiconazole) (1.0 l/ha) + Bravo (1.0 l/ha)		Inca (1.0 l/ha) + Bravo (1.0 l/ha)	Inca (1.0 l/ha) + Bravo (1.0 l/ha)
Proline (0.8 l/ha) + Bravo (1.0 l/ha)		Proline (0.8 l/ha) + Bravo (1.0 l/ha)	Proline (0.8 l/ha) + Bravo (1.0 l/ha)
Opus (1.0 l/ha) + Bravo (1.0 l/ha)		Opus (1.0 l/ha) + Bravo (1.0 l/ha)	Opus (1.0 l/ha) + Bravo (1.0 l/ha)
Folicur (1.0 l/ha) + Bravo (1.0 l/ha)		Folicur (1.0 l/ha) + Bravo (1.0 l/ha)	Folicur (1.0 l/ha) + Bravo (1.0 l/ha)
Proline (0.8 l/ha) + Bravo (1.0 l/ha)		Opus (1.0 l/ha) + Bravo (1.0 l/ha)	Caramba (metconazole) (1.0 l/ha)
Proline (0.8 l/ha) + Bravo (1.0 l/ha)		Opus (1.0 l/ha) + Bravo (1.0 l/ha)	Caramba (1.0 l/ha)+ Bravo (1.0 l/ha)
Opus (1.0 l/ha) + Bravo (1.0 l/ha)		Opus (1.0 l/ha) + Bravo (1.0 l/ha)	Caramba (1.0 l/ha)
Opus (1.0 l/ha) + Bravo (1.0 l/ha)		Opus (1.0 l/ha) + Bravo (1.0 l/ha)	Caramba (1.0 l/ha)+ Bravo (1.0 l/ha)
Proline (0.8 l/ha) + Bravo (1.0 l/ha)		Venture (1.5 l/ha) (bosclaid + epoxiconazole	Caramba (1.0 l/ha)
Untreated			

Despite difficult spraying conditions the treatments were applied at or very close to the ideal time, although due to the repeated rain events most leaves would have been infected by septoria before they were fully emerged. Disease pressure was extremely high at Knockbeg with the untreated plots having 91% Septoria on the flag leaf and 99% on leaf 2 shortly after the T3 application. Whilst disease pressure was not as bad at the Co. Meath site high levels of disease were present with 57% and 75% infection of the flag leaf and leaf 2 respectively around the same time as the assessments were conducted in Knockbeg.

The performance of the triazoles differed significantly between the sites, reflecting the differences in sensitivity of the septoria strains present when tested in the laboratory. At the Meath site which had a higher frequency of Septoria isolates with reduced sensitivity to prothioconazole and epoxiconazole, the disease control achieved and green leaf retention with both products was poorer than would have been expected in previous years (Fig 1 and 2) and to that achieved at the Knockbeg site. The poor performance of Folicur at both sites was not unexpected as strains of septoria with the I381V mutation were present at both sites at the start of the season. The programmes with either Opus or Proline (±Bravo) at T1, followed by Opus (± Bravo) at T2 and Caramba at T3 provided the best disease control, green leaf retention and highest yields at both sites.

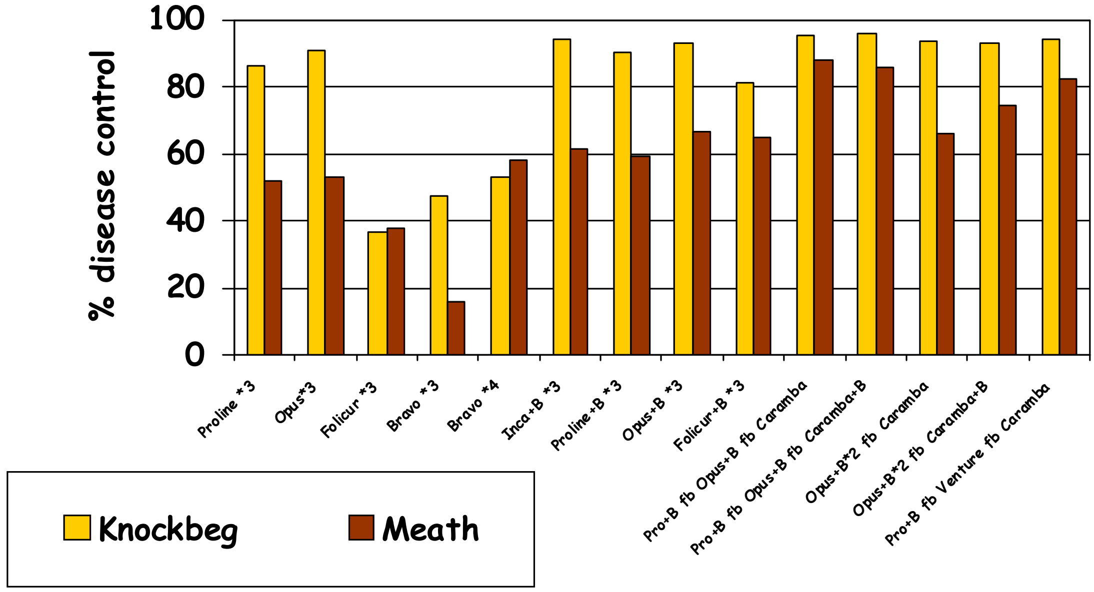


Fig 1 Percentage disease control on the flag leaf and leaf 2, at Knockbeg and Meath, assessed 3 weeks after the T3 application

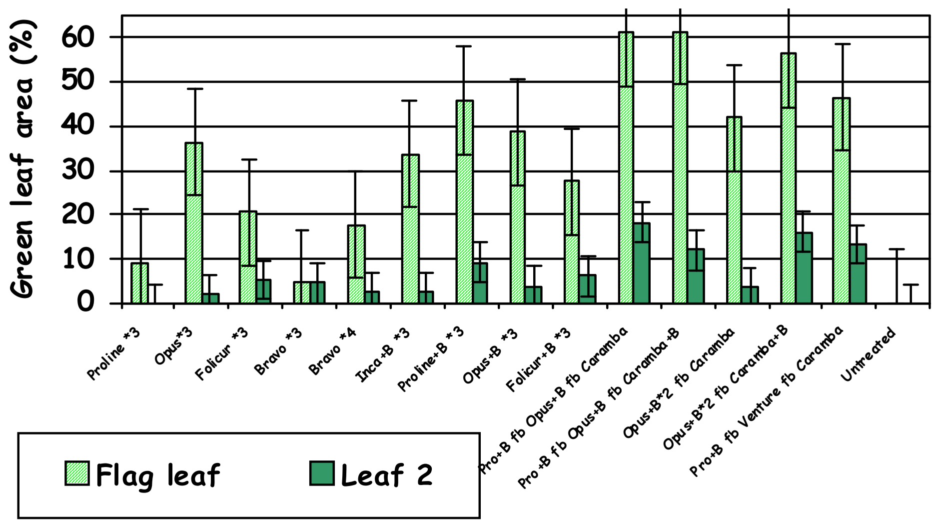


Fig 2 Green leaf area retention (% area) of the flag leaf and leaf 2, Meath, 22nd July 2009

It is difficult to be certain exactly what the implications of the new strains of septoria will have on disease control whilst the frequency of the new stains in the population is changing. However conducting the above programmes trials on two sites differing in the frequency of the new septoria strains gives us an indication. Clearly field performance of epoxiconazole and prothioconazole may be affected if these strains were to increase to the levels detected at the site in Co. Meath. Fortunately during the 2009 selection for these strains to the levels found in C. Meath only occurred at a handful of sites, although at most sites tested there was a small increase in the occurence. Although this is positive it does present a problem….“How do I know if I have got these strains in abundance in my crop?”. In reality it is not feasible (neither physically nor economically) to sample all crops. What the above trials demonstrate is that good disease control can be achieved irrespective of septoria population as long as an anti-resistant strategy aimed at minimising selection whilst maintaining disease control are applied.

The role of fungicides in yield formation in barley

Achieving a high yield of barley is highly dependant on producing a crop with a large number of grains per m^2. Research in the UK and elsewhere indicates that 85% of the variation in barley yield between sites and years is explained by differences in grain number. Differences in grain filling therefore account for only a small proportion of yield variation which indicates that in most cases the crop is able to fill the available grain capacity.

There is limited scope to increase the number of grains per ear in barley. In response to low plant population increases in grain number per ear of 70% have been recorded in wheat, but in barley the figure is usually closer to only 20-30%. The potential to increase ear number in barley is much greater with increases of over 5-fold being recorded in low plant populations.

Given low cereal prices one of the few options available to the grower to try and ensure at least some profitability is to maximise output for the minimum cost. In order to achieve this we need to target the spend on inputs where they are going to give the biggest return.

Fungicide timing

Given the importance of ear number to yield in barley, to maximise yield we need to ensure that shoot production is maximised and shoot death minimised. Fungicide research in both winter and spring barley in the UK in recent years has shown much larger than expected responses to early, and in the case of winter barley, even autumn sprays.

These UK experiments were targeting Rhynchosporium but the full range of diseases occurred across the sites and years. In winter barley autumn sprays gave a yield response of between 0.1 and 0.82 t/ha (average 0.46), T1 sprays between 0.02 and 0.96 t/ha (average 0.69) and T2 sprays between 0 and 1.12 t/ha (average 0.56). Similar results were found in spring barley with T1 sprays giving between 0.76 and 1.22 t/ha (average 1.04) and T2 sprays giving between 0.27 and 1.41 t/ha (average 0.85). The response to the early sprays could be explained through additional green canopy retention or formation prior to ear emergence; the resulting increased light interception relating directly to increased grain number per m^2.

2009 spring barley trials

In order to test if similar responses to early season disease control were likely to occur under Irish conditions, two spray timing experiments were set-up in spring barley in 2009 at Knockbeg, Co. Laois and Kildalton Agricultural College, Co. Kilkenny. All sprays were applied as 0.6 l/ha of Fandango; there were a total of 6 spray timings at roughly weekly intervals with treated crops receiving between one and six sprays between the start and end dates to produce different period of complete disease control. The number of sprays applied is not intended to be commercial option. The crops were slightly more backward than normal and the fourth spray timing (28th May) coinciding with GS32 and the sixth spray timing (19th June) with ear emergence. Disease levels were not high at either site although some Rhynchosporium came into the Kildalton site during stem extension. Disease levels at Knockbeg were lower than at Kildalton with some Net Blotch coming in very late in the season during grain fill. Of greater importance at Knockbeg was severe brackling and lodging which was significantly reduced and delayed by the late sprays.

Delayed necking and brackling in 3-spray treatments applied from traditional T1-T2 timings compared with the untreated.

Untreated yields at Knockbeg were poor at less than 5 t/ha (2t/ac), the response to fungicide was high with the best treatments yielding nearly 9 t/ha (3.6 t/ac). This large yield response was largely a result of the reduced brackling, and at Knockbeg it was a 3 spray programme with the earliest treatment at the normal T1 timing (28th May) and the latest at the normal T2 timing (19th June) that gave the highest yield, it would be expected that 2 sprays at T1 and T2 would have given similarly high yields (Figure 1).

At Kildalton, where there was slightly more disease, the highest yield was achieved with sprays starting on the 7th May and stopping on the 5th of June. These timings are both significantly earlier than the normal T1 and T2 timings for this crop, with the first spray applied before the start of stem extension and the last prior to ear emergence.

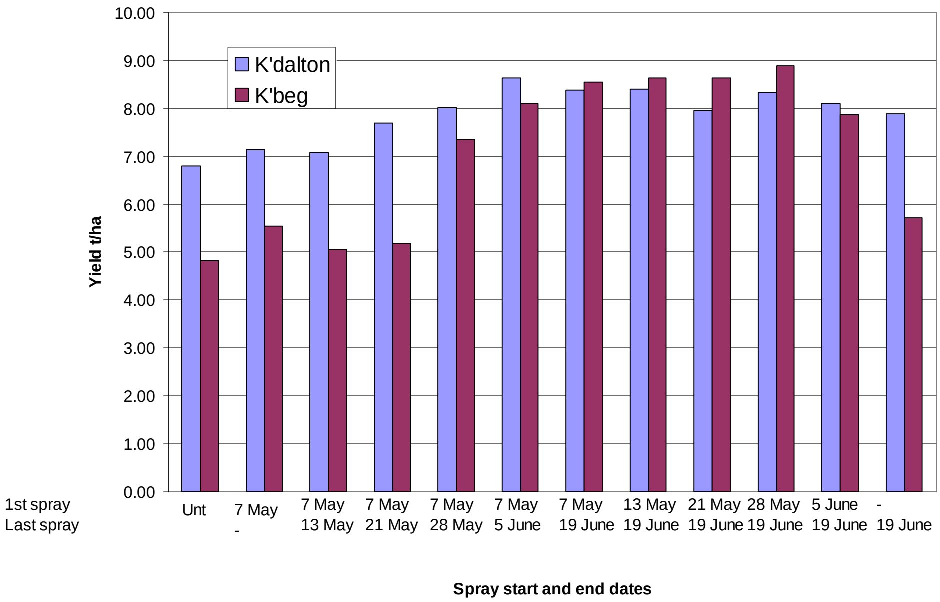


Figure 1. Yield t/ha @85% DM for a range of fungicide programmes with between 1 and 6 sprays on treated plots, the dates denote the timing of the first and last spray dates with treatments applied at roughly weekly intervals.

As in the UK trials, at Kildalton the highest yields were achieved with the highest grain number per m^2 and high grain number was mainly achieved through maintaining a high ear number per m^2 (Figure 2).

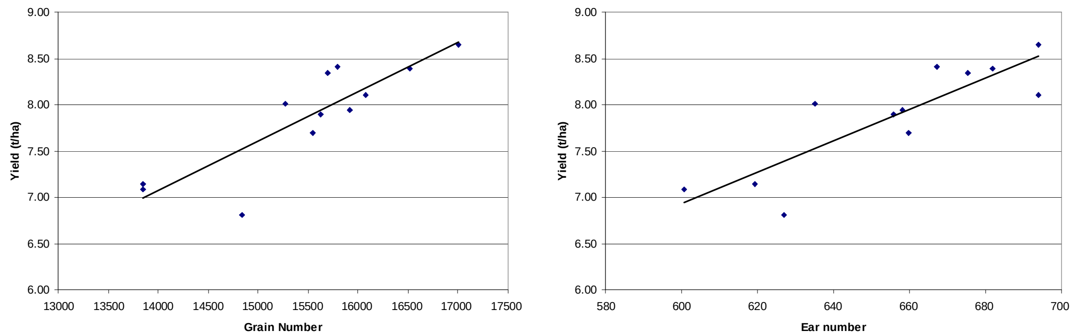


Figure 2. Relationships between grain number per m^2 and ear number per m^2 at Kildalton

Field trials of the oilseed crop *Camelina sativa* and properties of the obtained oil

Frohlich A. **RMIS No. 5771**

The stabilisation of camelina oil was evaluated with nine synthetic and eleven natural pure and formulated antioxidants and antioxidant extracts at four and six different concentrations respectively. The effect of stable oils, namely coconut and palm oils on the stability camelina oil was also investigated using 10, 15 and 20 % blends. Thermal induction times determined by the Rancimat method (110^{o}C, 10lt/hr airflow) were used for the initial determination of stabilising effect. The method is rapid and reproducible and also it is widely used by the food industry for the determination of oil stability. In order to determine the effect of antioxidants on the oxidation process accelerated storage (65^{o}C, no airflow) of camelina oil was carried out with periodic monitoring of six oxidation indicators, namely peroxide, diene and triene contents, p-anisidine values, tocopherol deactivation time and changes in fatty acid profiles.

Most synthetic and natural antioxidants showed stabilising effects by increasing thermal induction times of camelina oil, although in some cases the observed effect was relatively small. The antioxidants in order of increasing thermal induction times (% increase over camelina oil in parenthesis) included the natural antioxidants α-tocopherol (0), δ-tocopherol(0), tocopherol mixture(12), rosemary extract(53), green tea extract(76), rosemary-gallic acid(86), rosemary-tocopherol-ascorbyl palmitate(96), carnosic acid(135), EGCG(epigallocatechin gallate)(152) and EGC(epigallocatechin)(198) and the synthetic antioxidants ethoxyquin(0), BHT(butyl- hydroxytoluene)(5), BHA(butyl hydroxyanisole)(6), BHA-BHT (14), PG(propyl gallate)(103), BHA PG blend(107), TBHQ(t-butyl hydroquinone)-PG (162), TBHQ(257) and TBHQ-citric acid (337). The natural antioxidants EGC, ECGC, carnosic acid, rosemary extract-gallic acid, and the synthetic antioxidants TBHQ and PG and their blends increased thermal induction times above that of sunflower oil. Only the synthetic antioxidant TBHQ and its formulation with citric acid increased the thermal induction time of camelina oil above that of rapeseed oil. Tocopherols, BHT, BHA and ethoxyquin had relatively small or no effect on the stability of camelina oil.

Addition of stable oils, namely coconut and palm oils to camelina oil at the rates of 10, 15 and 20 % resulted in a relatively small increase in stability of camelina oil, but addition of TBHQ, tocopherol rosemary blend and BHT to camelina with 20 % coconut oil increased thermal induction times by 20-80 % when compared to pure camelina oil with the same antioxidants. Palm oil was less effective.

The antioxidants had a consistent effect on the six monitored oxidation indicators. Rosemary-tocopherol-ascorbyl palmitate and TBHQ-citric acid blends stabilised camelina oil for five and twelve days respectively and all oxidation indicators remained constant during that period. However when propyl gallate and rosemary extract were used, there was a small increase in the levels of all oxidation products in the first three to four days, and a much more rapid increase in each case afterwards. BHA and δ-tocopherol had no significant effect on the rate of formation of oxidation products or on the rate of oxidation of susceptible components during accelerated storage. Results obtained so far indicate that camelina oil stabilised with TBHQ-citric acid or rosemary-tocopherol blends can be more stable than either rapeseed or sunflower oils, if the oxidation indicators monitored in the present work is used as criterion of oil stability. Similarly the stability of camelina oil can be increased above that of sunflower oil with appropriate amounts of PC or rosemary extract.

The organelle genomes of perennial ryegrass: Sequence discovery and genomics for basic and applied agricultural research

Barth, S. **RMIS No. 5532**

The complete mitochondrial genome of *L. perenne* was sequenced, and partially assembled and annotated. The complex nature of plant mitochondrial genomes prohibited the complete assembly and annotation. 24 contigs have been annotated. Nevertheless, the availability of sequence information for the mitochondrial genome is of great value as *Lolium* is of one of the most important forage grasses of the northern hemisphere. Only 24 plant mitochondrial genome sequences are publicly available to date and although ten of them are from grass species (with a strong bias to Panicoideae species) no forage grass species had until now been sequenced. Furthermore, the number of published articles on sequenced genomes is very restricted and thus background information to the published mitochondrial genomes only available for *O. sativa*, *T. aestivum* and *Z. mays*. Although the final order of mitochondrial genes within the *L. perenne* mitochondrial genome could not be established, conserved regions across many Poaceae species that might be suitable for future population and phylogenetic studies were detected. During the assembly of the *L. perenne* mitochondrial genome also microsatellite regions could be detected. These regions might be of interest in future studies as additional tools for population and phylogenetic studies. Also insights into the extent of intracellular gene transfer from the chloroplast genome to the mitochondrial genome were gained. Once mitochondrial genome sequences of *L. perenne* endophytes become available it will be interesting to see to what extent horizontal gene transfer can be observed in *L. perenne*. For the future it would be also interesting to analyze the complete mitochondrial genome for further open reading frames that might be involved in the cytoplasmatic male sterility (CMS) mechanism.

Generation of mapping tools, construction of suitable plant material and isolation of agronomic traits in *Lolium perenne* L.

Barth, S **RMIS No. 5244**

Self-incompatibility (SI) was defined as the inability of fertile plants to reproduce after selfing. Work is ongoing on the fine mapping of the two major loci, S and Z. Moreover, in addition to the S and Z components determining SI, other genetic factors are assumed to be involved in the signal cascade leading to the SI response. Additional loci, T and F have been identified. There is evidence of association between the S locus and a locus (F) on linkage group 3 linked to the isoenzyme glutamate oxalo-acetate transminase GOT/3 (Thorogood *et al.*, 2002), in ryegrass. Additionally, segregation distortion has been observed in an F2 perennial ryegrass mapping family on chromosome 5 which indicates that another locus is involved in SI in *L. perenne*, which is probably equivalent to the *Secale* S5 and *Phalaris* T loci (Thorogood *et al.*, 2005). This distortion is due to segregation of a self-compatible locus, T. For these two further SI loci mapping work has been started making use of comparative genetic tools. The SI work is in collaboration with Dr. Daniel Thorogood at IBERS/Wales.

Genomics of the biomass crop Miscanthus: characterizing organelle genomes and assessing nuclear polyploidy variation

Barth, S. **RMIS No. 5763**

The genus *Miscanthus* belongs to the family Poaceae and has a wide distribution extending from south-eastern Asia, through China, Japan and Polynesia, with few species also in Africa. Most of the Miscanthus species are capable of great biomass growth in a wide range of climatic conditions and are suitable for use as alternative energy source. Very few *Miscanthus* genotypes have been tested for their biomass potential and among them, one species, *Miscanthus* x*giganteus*, has been grown in trials in Ireland for more than 15 years. This is a sterile triploid with a high yield potential and a low invasiveness risk due to its sterility. However to date genomics tools in this species are still rather limited.

A collection of 133 different *Miscanthus* genotypes was obtained from different sources and planted in two replications in the field in Oak Park. During the 2008 and 2009 growing seasons, morphological characters have been scored. Herbarium specimens collected from various international herbaria have also being screened to measure characters in the full range of *Miscanthus* species that have been described in the literature.

DNA of a *M. sinensis* clone was used for the construction of a library enriched with nuclear microsatellites (SSRs). The SSR library with 192 clones has been sequenced. Clones containing useful SSRs have been selected and primer pairs for 80 SSRs from the available sequences designed. These primers have been used to amplify, through PCR, regions of the nuclear *Miscanthus* genome containing TCn, TGn and GATAn repetitions. 22 potentially polymorphic primer pairs have been used to genotype our *Miscanthus* collection.
Crosses between genotypes of different ploidy levels have been successfully done. Leaf material of the offspring will be used to test maternal inheritance of the chloroplast genome using our previously developed chloroplast SSRs.

Examining the genetic response of maize to low-input and low temperature conditions

Barth, S. **RMIS No. 5769**

Maize is the world’s most widely grown cereal due to its high economic value and its ability to grow in a huge range of environments. In Ireland the environmental conditions are marginal for the crop and especially early development is impacted by cold soil temperatures. Twelve maize varieties with known difference in cold tolerance were germinated under a range of different temperatures and root and shoot growth parameters were determined. A series of physiological experiments was carried out for the initial stages of development following germination in micro climate growth chambers under control and chilling conditions. The physiological response of the tested maize has been characterized. From these trials RNA from roots and shoots was collected for probing a custom made chilling stress macro-array. This custom array was developed in Dr. Thomas Gallagher’s lab in UCD. Work is in progress to utilize this macro-array for an initial analysis of gene expression under chilling conditions.

Developing advanced technologies and resources for conventional and “-omics”-based white clover breeding strategies

Milbourne D., Mehenni-Ciz J., and Barth S. **RMIS No. 5762**

The International Trifolium Network (ITN) is an international consortium of laboratories and research groups whose goal is to develop community-accessible genomics based resources for genetics and breeding of clover (Trifolium) species. As part of our contribution to this initiative, we are in the process of developing a large single nucleotide polymorphism (SNP) database for white clover, to allow the easy and routine development of genetic markers in this species. Our goal is to use the SNP markers for linkage disequilibrium mapping studies in a white clover association mapping population, comprising 408 half-sib families, that was developed for the ITN by Dr Derek Woodfield, AgResearch New Zealand. In addition to the ongoing global SNP analysis, we are currently identifying SNP polymorphisms in specific genes which may be candidates for involvement in key traits important in white clover.

We extracted RNA from leaf, petiole and flower parts of an inbred line of white clover that was previously developed at IBERS. This sample was subjected to 454 Titanium sequencing on one half of a picotitre plate on the Roche GS-FLX sequencing platform. The sequencing run produced a total of 750,000 transcript fragment reads with an average length of ~400 nucleotides. To reduce the redundancy in this dataset, the sequences were clustered using the CD-HIT algorithm, at a similarity threshold value of 90%, resulting in a reduction of the dataset to approximately 250,000 sequence clusters.

To develop a “test” dataset in which potential single nucleotide polymorphisms could be identified, we chose 17 genetically diverse individuals from the ITN association mapping population, extracted and pooled RNA from leaf and petiole material of these genotypes, and subjected them to Solexa sequencing on a single channel of the Illumina GAII sequencing platform. This sequencing run generated approximately 14.5 million reads of an average length of 76 nucleotides.

In order to identify potential single nucleotide polymorphisms amongst the 17 genotypes subjected to Solexa sequencing, the 14.5 million reads from this sequencing run were assembled against the 250,000 454-Titanium sequences derived from the inbred line, using the Lasergene package from DNASTAR. Global analysis of this dataset is ongoing, but preliminary analysis reveals that extensive putative SNP polymorphism can be revealed by this approach (Figure 1).

White clover is an allotetraploid, thought to have evolved originally by the hybridisation of two ancestor species today best represented by the species *Trifolium occidentale* and *Trifolium pallescens*. This allotetraploid status is well conserved in *T. repens*, with the haploid genome consisting of 8 homeologous pairs of chromosomes. The existence of highly conserved, but independently segregating homeologous sequences is of evolutionary interest, but may also be problematic for SNP development in white clover. Interestingly, many sequence contigs (with sufficient sequence depth) in our test:reference sequence alignment contain biallelic SNP alleles present at an approximate 1:1 ratio. We posit that these particular SNPs represent the two homeolog types for these genes. The ability to identify and differentiate between these homeolog-specific SNPs will be important for SNP marker development in white clover.

Currently we intend to test the association between SNPs in candidate genes for traits such as digestibility, carbohydrate content and abiotic stress tolerance in the ITN association mapping population in order to both develop genetic markers, and further understand the biology of these traits.

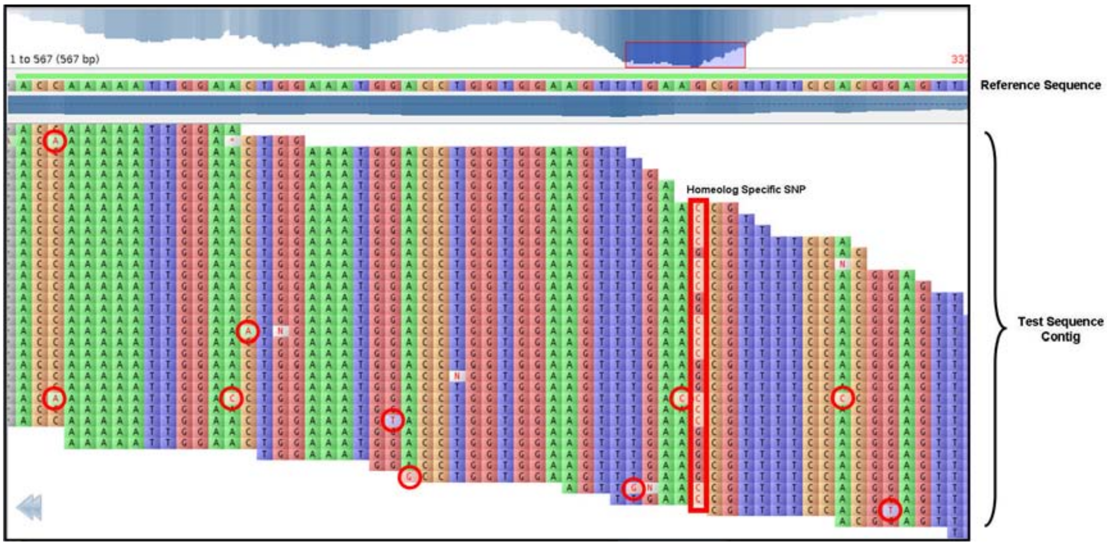


Fig.1. A graphical overview of part of a sequence contig (homologous to the *C4H* gene from *Arabidopsis thaliana*) comprising 87 Solexa reads from the "test" library, aligned against a single read from the "reference" library generated using 454-sequencing.

Several single nucleotide polymorphisms (circled in red) are visible amongst the "test" sequences, and these represent potential SNP variation between the 17 genotypes originally used to create the "test" library. An apparently biallelic SNP putatively differentiating the two highly similar homeologs present for this sequence in allotetraploid white clover is indicated by a red box**.**

Developing a knowledge-base for the biotechnology-driven exploitation of self-incompatibility in white clover breeding

Milbourne D, Casey N. and Barth S. **RMIS No. 5761**

White clover (*Trifolium repens* L.) has a strong self-incompatibility system preventing self fertilisation. The molecular basis of self-incompatibility in *T. repens* is unknown, but it is under the control of a single locus, which is expressed gametophytically. To locate the self-incompatibility locus (S locus) in *T. repens*, we carried out cross-pollination experiments in an F1 mapping population derived from a cross between two divergent parents and constructed a genetic linkage map using 64 white clover SSRs and 339 AFLP markers.

The female parent map comprised 17 distinct linkage groups, spanning 1,477 centimorgans (cM) and ranging in size from 44 to 145 cM. This map contained alleles of 48 SSR markers and 196 AFLP markers, resulting in an average map density of 5.7 markers per cM. A single morphological marker (the white V-shaped leaf mark) was also placed on the female parental map at a distal region of homoeologue group 5 (G). The male parent map comprised 14 linkage groups spanning 1135 cM, ranging in size from 37 to 128 cM. This map contained alleles of 43 SSR markers and 146 AFLP markers with an average map density of 5.7 markers per cM. Approximately 14% of markers exhibited segregation distortion on the paternal map; 23 marker loci, 22 SSRs and one AFLP, were shared between the two parental maps. The presence of SSR markers from previously published maps of white clover on the parental maps allowed the identification of linkage groups (LGs) belonging to the eight homoeologue groups (HGs) of white clover. For the female parental map, it was possible to assign 15 of the 17 LGs to one of the eight white clover HGs A-H (as per the nomenclature of Barrett et al. 2004) or 1–8 (George et al. 2008), each HG being represented by two LGs with the exception of HG 2 (F) that was represented by a single LG. Two further LGs remained unassigned because they consisted entirely of AFLPs. For the male parental map, 13 LGs were assigned to HGs, with HGs 5 (G), 6 (H) and 8 (B) being represented by a single identifiable linkage group. One further LG, consisting entirely of AFLPs, remained unassigned.

The S locus was mapped in both parental maps to individual linkage group members of HG 1 (E) (Fig. 1). When an interim round of analysis revealed HG 1 (E) was the likely genetic location for the S locus, a concerted effort was made to include a reasonable number of HG 1-specific SSRs in the largely AFLP-based map to order and orient these groups comprehensively. As a result, alleles of 9 HG 1 (E)-specific SSR markers in total have been used to identify and orient the individual parental LGs constituting HG 1. The orientation of the linkage maps shown in Fig. 1 (in which the S locus is mapped to the distal end of HG 1) is presented as in Barrett et al. (2004).

This study provides molecular evidence for the first time that the control of gametophytic self incompatibility in *T. repens* involves a single functioning S locus. This result is in agreement with previous data from cross-pollination studies showing a pattern of cross-incompatibility consistent with a single locus under gametophytic control. This work is more fully described by Casey et al (2010). Further work will focus on attempting to identify the genes underlying the control of SI at this locus in white clover.

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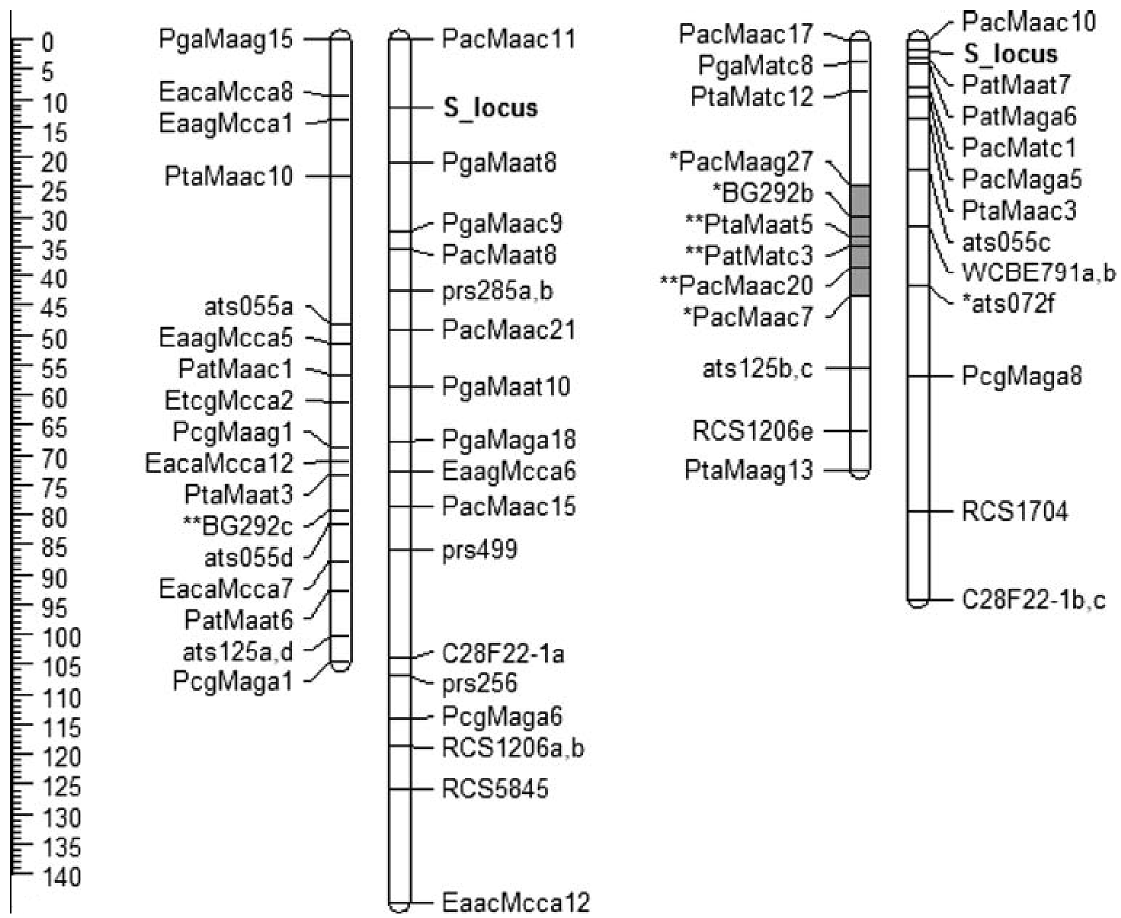


Fig.1. Linkage maps of homologue 1 (E), S1S4 (left) and R3R4 (right). Scale (at left) is in centimorgans (cM). Asterisks segregation distortion (declared at P\0.05). Grey shading area is defined by distorted markers. The S locus is shown in bold

Sequencing potato chromosome IV

Milbourne, D. **RMIS No. 5744**

Teagasc Crops Research Centre is a member of the Potato Genome Sequencing Consortium (PGSC), an international group of academic and industrial organisations committed to sequencing the complete potato genome ash to meet contributing the world's food needs. On the 23rd of September 2009, the consortium released the first draft of the potato genome into the public domain. This first draft of the genome was produced in a doubled monoploid potato genotype referred to as DM1-3 516R44 (DM). The sequence was based on a shotgun sequencing strategy using the Solexa next generation sequencing (NGS) platform from Illumina. Currently the draft sequence is being improved, with revised assemblies incorporating BAC-end sequence information and 8kb and 20kb paired-end reads using the 454 next generation sequencing platform from Roche (Teagasc has contributed considerably to the latter). The goals for the final published version of the draft sequence (expected in 2010) are:

- Identification of >95% of genes plus regulatory regions in potato
- Coverage of >95% of ESTs (>250 bp, 10 Ns)
- >50% of the genome anchored to chromosome (that should contain 80% of the genes)
- Provision of a set of annotated genes
- Development of defined portals for public access
- N50 contig size > 15 kb
- N50 scaffold size > 0.5 Mb

In addition to direct contributions to the sequencing, Teagasc has contributed to the anchoring of the genome sequence by analysing the segregation of 50 microsatellite markers derived from the genome sequence in a genetic mapping population constructed using the genotype DM as one of the parents. Teagasc has also contributed to the assesment of the quality of the draft assembly by resequencing several regions of the genome using the more traditional Sanger-based approach upon which most genome sequencing projects have been based to date. At Oak Park, we continue to work on characterising the major disease resistance locus on chromosome 4, and have now produced a high quality sequence encompassing a region of one million nucleotides, which contains an extensive resistance gene cluster.

Enhancing management practises to maintain the sustainability of current potato systems

Mullins E. **RMIS No. 5772**

Funded by the DAFF-RSF programme

The potential introduction and adoption of genetically modified potato crops in Europe, such as the recent authorisation for the cultivation of BASF Amflora starch potato across the European Union, has raised concerns for both growers and consumers in regard to the feasibility and effectiveness of current coexistence measures. At present there is no legislation in Ireland preventing the cultivation of GM crops, thus once a GM variety is commercially placed on the EU common catalogue; Irish growers can obtain seed for cultivation. Therefore the development of a coexistence strategy is critical to protect the genetic integrity of non-GM potato crops and give growers the freedom to cultivate both conventional and GM varieties in parallel cropping systems.

Critical to achieving this is the necessity to determine the degree of pollen-mediated gene flow between two potato crops. Field trials were completed at Oak Park across two independent field sites. The male fertile (pollen donor) potato variety Désirée was sown in a 15m x 40m plot with a male-sterile (pollen receptor) variety (var. British Queen) planted in neighbouring 30m x 40m plots with a 5m isolation zone between the plots. The sowing of each variety was staggered by two weeks to ensure that flowering between the two varieties was synchronous. The frequency of flowering on the pollen receptor sub-plots was recorded twice weekly by noting and tagging the plants with at least one fully opened flower. As British Queen is male sterile, successful gene flow was determined by scoring for the presence of berries on receptor plants. In total 4 berries were collected, all of which came from one of the sites (Table 1). The maximum distance recorded for formed berries was 11m. The true potato seed within each berry was extracted and 22 F_1 individuals were sown in the glasshouse. The paternity of each individual was assessed using microsatellite analysis. STM 5130, 5136 and 5148 confirmed Désirée as the paternal donor in 20 of the 21 F1 hybrids confirming that this was as a result of pollen flow from the Désirée variety, sown in the centre of each site (Table 1).

Table 1. **Total number of berries and seeds harvested from British Queen plots**

Site	Potato variety	No. berries collected	Total seeds	Total No. Germinated Seeds	Distance from Désirée plot	No. Verified as F_1 Hybrid
College Field	B. Queen left	3	76	10	8-10 metres	9
	B. Queen right	1	29	11	11 metres	11
Church Field	B. Queen left	0	0	0	0	0
	B. Queen right	0	0	0	0	0

Following the evaluation of the current traditional potato production practices in 2008, two areas were identified as the main challenge points within the production chain for effective co-existence of GM and non-GM crops;

i) Traceability

Seed segregation and labelling both pre-sowing and post-harvest highlighted the problem of traceability of GM tubers throughout the production chain, thus a method to ensure traceability needed to be developed. The key elements which had to be considered when developing the labelling procedure were that it needed; i) to be cost effective, ii) give confidence and iii) similar to existing practices. Therefore the GM passport was modelled on the current seed potato label and the cattle identification card (Figure 1).

ii) Post-harvest emergence & persistence of volunteers on commercial sites

In October 2009, five commercial sites (~1ha) surveyed directly after harvest to assess tuber loss post-harvest. It was found that between 3.9 and 8.9 tonnes of tubers remained in the field post harvest, resulting in a loss of €780-€1780/hectare. The sites were either put into a Winter Wheat or Grass crop rotation. In June 2010, the sites were revisited to assess the emergence of volunteers. It was found that between 648 and 2730 volunteers/acre from the previous years potato crop had emerged. This highlighted the need for additional commercial sites, under different cropping systems, to be surveyed to ascertain the extent of the problem associated with potato volunteers. In total 30 fields located at 13 commercial sites have been surveyed under five different cropping regimes; Spring Barley, Spring Wheat, Winter Wheat, Spring Oats and grass Analysis of the results is currently underway to determine the impact of subsequent cropping on potato volunteer growth.

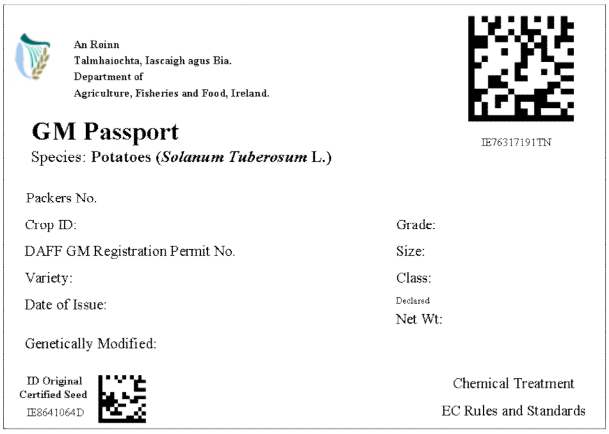


Fig.1. Model for the GM traceability passport

Fig. 2. The emergence of potato volunteers in Spring Barley

High value products and ethanol from wheat straw and bran: enhancing our understanding and capacity for fungal bioconversion.

Mullins E. **RMIS 5773**

The filamentous fungus *Fusarium oxysporum* is capable of breaking down lignocellulosic wheat straw and bran into sugars and subsequently converting them into ethanol in a single simultaneous step. Despite having a low fermentation rate, this process of simultaneous lignocellulosic saccharification and fermentation (SSF) is considered a major advantage over other microbial fermenters providing an efficient and cost-effective alternative with both agricultural and environmental benefits.

The overall aim of this project is to obtain an increased understanding of fungal bioconversion and to enhance the ability of *F. oxysporum* to produce bio-alcohols.

The first task was to generate a library of gene disrupted mutants using *Agrobacterium tumefaciens* - mediated transformation (ATMT). A parent strain characterised as a good ethanol producer was selected for mutagenesis and parameters including hygromycin sensitivity, spore concentration and co - cultivation time were optimised to obtain an efficient and robust transformation system utilising the *A. tumefaciens* strain AGL1 harbouring the binary vector pSKI834 conferring hygromycin resistance and expressing enhanced green fluorescent protein (eGFP).

The second task was to screen the mutants generated from the previous task for increased or decreased ethanol tolerance. A high through put assay was developed to screen the mutants for tolerance to two solvents: ethanol and butanol. The ability of the parent strain to tolerate both alcohols was assessed and suitable solvent concentrations were then selected for use in the screen. In addition, the screen was adapted to the ATMT process allowing for the simultaneous generation and screening of mutants.

A total of 1,587 mutants have been generated and screened for solvent tolerance traits. From this population 182 mutants have been selected as candidate strains and categorised as either hyper-ethanol (*n=36*) or hyper-butanol (*n=96*) tolerant strains or hyper- tolerant to both solvents (*n=50*). These candidate strains are being purified by single spore isolation to obtain pure mono-conidial cultures for long-term storage and further analysis.

Predicting the impact of genetically modified (GM) cropping on Irish biodiversity

Mullins E. **RMIS: 5621**

Funded through the STRIVE programme of the EPA.

To be in a position to describe the impact of a range of different GM crops/traits on Irish biodiversity, it is first necessary to identify those GM crops that could be suited to Ireland's tillage industry over the next 20 years against a backdrop of future agricultural challenges and alternative management practices. These future macro-challenges to the Irish agri-environment include (but are not exclusive to) climate change, increased environmental legislation (e.g. EU Water Framework, Nitrates Directive, proposed reform to the Pesticide Directive and Common Agricultural Policy reform), mitigating biodiversity loss and sustainable biofuel production.

The goal of this study was to collate a register of GM traits such that a list of potential GM crops could be prioritised against the backdrop of the challenges facing the tillage sector. Clearly, the crops with the most significant potential for genetic modification are those that are grown widely and/or receive high applications of pesticides and fertilisers (e.g. potato, wheat, barley and maize). GM traits with significant agronomic potential include late blight resistant potato, *Fusarium* head blight resistant wheat and Septoria resistant wheat and herbicide tolerant winter oilseed rape and maize. Following on from these, crops with enhanced nitrogen-use efficiency could provide significant input to the tillage sector in light of EU-based restrictions on nitrogen usage, crops with elevated protein content could offset the costs of imported animal feed and crops with modified oil content/lignocellulose composition could assist in biodiesel/bioenergy production at a regional level.

In addition, the potential biodiversity impact of two GM crops that are most likely to suit Irish agronomic conditions was also examined. These are glyphosate or glufosinate herbicide tolerant (HT) maize and oilseed rape and following an extensive analysis of the scientific literature on the subject several key conclusions were made:

- There are many 'wild' species related to oilseed rape in Ireland, none of which are native and many of which are highly unlikely to survive when crossed with the current oilseed rape crop plant (*Brassica napus*). The only exception is wild turnip (*Brassica rapa*), which is an earlier oilseed crop now no longer farmed but anecdotally present in marginal habitats. Worldwide, there have been ongoing and intensive surveys of the potential for GMHT *B. napus* to transfer herbicide tolerance to *B. rapa*. While it has been shown that this will indeed occur, the primary issue remains the consequence of this gene flow event; that is, what will happen to the resulting offspring? Critically, in the absence of a selection pressure (spraying with the herbicide for which they have a tolerance), these GM hybrid individuals have no physical advantage to their non-GM neighbours. As they also contain a significant portion of a crop genome they will not have the competitive ability that weed populations possess and will therefore not survive over time. In Ireland, marginal habitats are not routinely sprayed so it can be concluded that GM hybrids with an herbicide tolerant trait will not proliferate and spread. Separately, there is no likelihood of maize impacting on wild relatives as none exist in Ireland.

- In real-world conditions, there are some scenarios where accidental spraying may occur and where management arrangements may give rise to an opportunity for a GMHT plant to prevail in the landscape. This was examined and presented in a series of five hypothetical scenarios. It was shown that there are no credible scenarios where a GMHT crop can persist or prevail over time any more than a non-GM crop, outside of the confines of a managed field environment. Furthermore, it is also shown that it is in the management of the GM or non-GM crop that the potential for biodiversity impact is at its greatest.

- Glyphosate and glufosinate toxicity was examined in detail and it was concluded that these two compounds have significantly lower toxicity than those compounds currently in use across conventional systems. Using a recently-developed index of biodiversity impact (CINMa), the two GMHT crops were subjected to an analysis of their potential for impact. It was shown that in the management of GMHT maize there is the potential for benefiting landscape biodiversity by lowering toxic materials and management frequency. The same may be said for oilseed rape management, but there is some likelihood for transfer of genetic material to a wild relative. The potential impact of this is low and there is a net beneficial impact as with maize..

Producing Biomass for Energy

Finnan, J. **RMIS No. 5788**

The use of biomass as a source of energy forms a significant part of government strategy to reduce fossil fuel dependence and to mitigate the impact of climate change. However, energy crops and short rotation coppice have not been grown in this country to any significant extent and relatively little research work has been carried out in Ireland.

The objectives of this study are to:

- Evaluate the potential of a range of energy grasses in Ireland
- Evaluate the effect of agronomic factors on the use of Hemp as a bioenergy feedstock
- Evaluate the biomass potential of new willow varieties
- Determine the optimum method for harvesting and drying whole stem willow
- Quantify *Miscanthus* harvesting losses and investigate the feasibility of reducing these losses by modifying existing machinery.

An evaluation of perennial grasses for Bioenergy

Energy crop trials were established during May 2008 at three sites; Oak Park, Knockbeg and at Johnstown Castle. The trials contained the following grasses:

- Miscanthus
- Reed Canary Grass (varieties Bamse and Chiefton)
- Switchgrass (varieties Kanlow and Shawnee)
- Tall Fescue (variety Olga)
- Cocksfoot (variety Sparta)

Switchgrass growth was poor during the first two years growing seasons. One variety (Kanlow) did not survive the first winter. During 2009, Yields of reed canary grass, cocksfoot and tall fescue are shown below, all of these grasses received 60 kg N/ha during 2009. The yields of these grasses averaged 8tonnes of dry matter per hectare, yields of reed canary grass were slightly higher when compared to yields of cocksfoot and tall fescue.

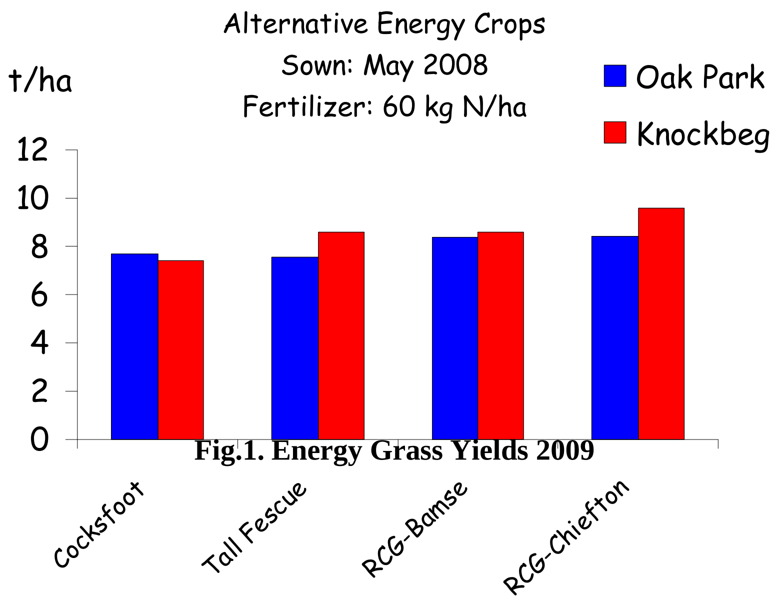


Fig.1. Energy Grass Yields 2009

An evaluation of hemp for bioenergy

Previous research at Oak Park proved that Hemp could produce high yields of stem dry matter in the Irish climate. Hemp has begun to attract interest as an energy crop both as a combustible material and as a feedstock for second generation ethanol since hemp fibres have a high cellulose content, 135 ha of the crop were grown in Ireland during 2008.

Hemp can be grown in Ireland without the use of pesticide. Consequently, the main input is fertilizer and nitrogen fertilizer if levels of P and K in the soil are high. Trials continued in 2009 to determine the optimum strategy for applying nitrogen to hemp in order to optimise both the energy ratio and the economics of the crop. The objective of the trials in 2009 was to determine if the nitrogen fertilizer requirements of Hemp could be reduced by altering the time of fertilizer application. Equivalent amounts of nitrogen were applied at sowing, at emergence and two weeks later and split between all three occasions. Highest stem yields, however, were obtained when all of the nitrogen was applied at the time of sowing.

Optimisation of Miscanthus harvesting

Miscanthus can be harvested using conventional agricultural machinery but harvesting losses can be as high as 30%. Work commenced during 2008 to improve carbon capture from Miscanthus. During 2009, the work focussed on the quantification of:

- harvest losses
- crop drying when cut at different dates and left to dry
- the effect of harvest machinery on subsequent growth

Results from experiments conducted in 2009 showed that harvested losses were lower when the crop was cut using a self-propelled forage harvester compared to systems where the crop was cut first with a conditioner mower before being baled (Figure 2). Time of harvesting experiments conducted during 2009 showed faster drying rates when the crop was cut in February and March and left to dry compared to the standing crop.

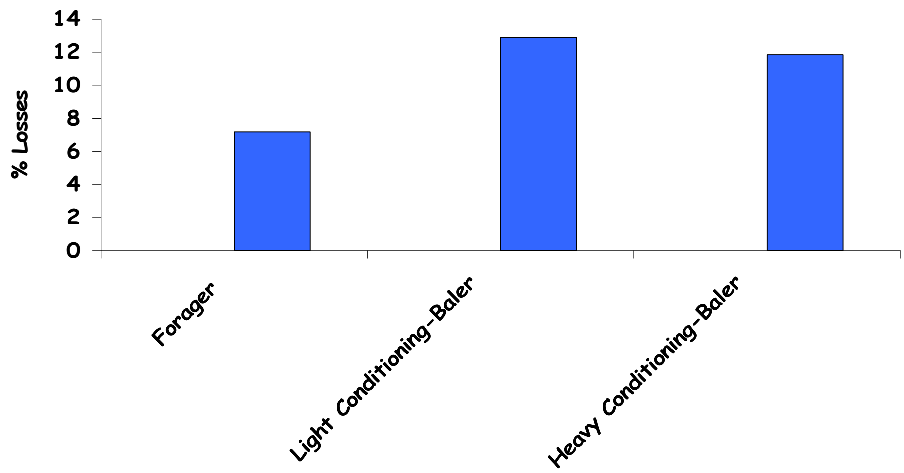


Fig.2. Miscanthus harvest losses from different harvesting systems. The three systems tested were a self-propelled harvester, a mower with light conditioning and a mower with heavy conditioning

Experiments were also conducted to investigate the effect of harvesting in wet and unfavourable conditions on subsequent growth. The experiments were conducted in wet conditions after the shoots had begun to emerge in order to simulate late harvesting under wet conditions. Results from the experiments (Fig 3.) show that both shoot numbers and shoot height were suppressed by harvest traffic under these conditions.

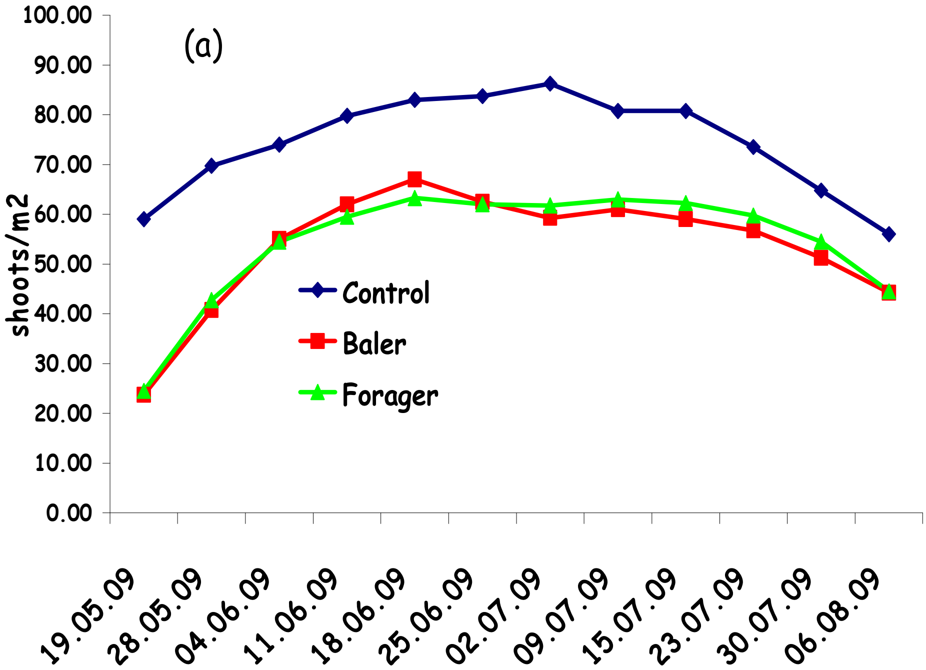
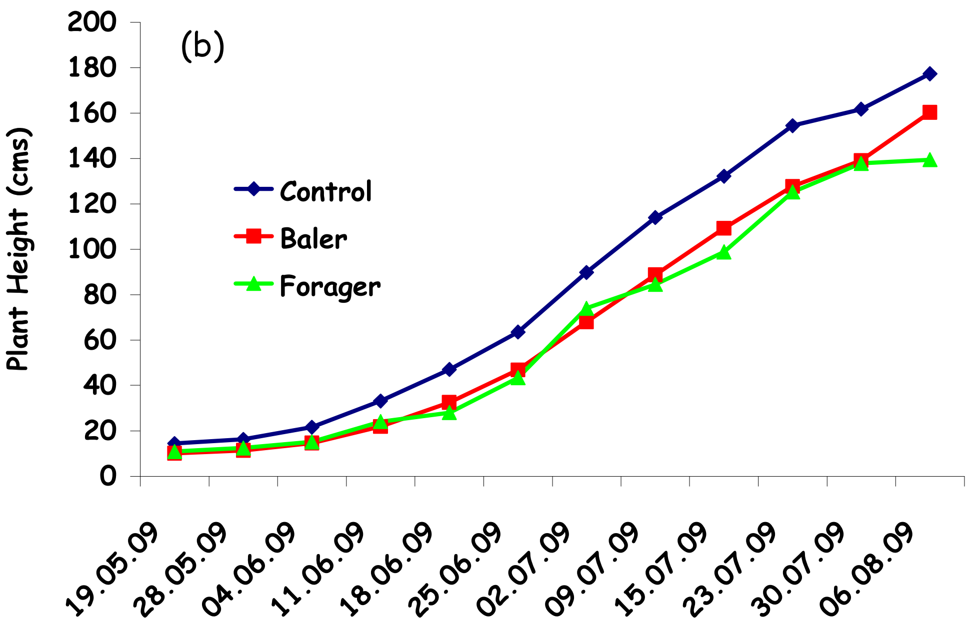


Fig.3. re- **Harvest traffic experiment. Effect of different harvest techniques on subsequent growth and plant height**



Evaluating Irish Grassland as a source of Bioenergy: Environmental Impacts and Long Term Sustainability

Finnan, J. **RMIS No. 5819**

Biomass will play an important role for the EU to reach its target for the replacement of fossil fuels with renewable energy sources. However, large-scale energy crop production requires major land-use changes and may cause adverse effects to the environment. In contrast, grassland biomass can be used as a feedstock for anaerobic digestion, a technique in which bacteria break down organic material to yield a gas which can be used as a fuel for the generation of heat and electricity or as a vehicle fuel. The waste product from anaerobic digestion, known as digestate, is rich in nutrients and is a valuable fertilizer.

Experiments began in 2009 at Oak Park to quantify the fertilizer value of digestate as well as the greenhouse gas emissions which arise from the application of digestate. The objective of this work is to improve the economics and the greenhouse gas balance of a grass to energy system by replacing conventional fertilizer with digestate. Corresponding amounts of nitrogen mineral fertilizer (calcium ammonium nitrate) and digestate were applied to grass plots at intervals of six weeks, measurements were made of biomass yield and greenhouse gas emissions.

Grass yields increased with application of increasing quantities of digestate or mineral nitrogen. (Figure 1). However, yield increases after digestate application were lower than those which received corresponding amounts of nitrogen applied as a mineral fertilizer. Greenhouse gas emissions were considerably lower in digestate treatments compared with treatments which received corresponding amounts of mineral nitrogen (Figure). This latter finding is very significant for the life cycle analysis of grass to energy systems as greenhouse gas emissions from fertilizer manufacture and field greenhouse gas emissions are reduced when digesate is used as a fertilizer in the place of mineral fertilizers.

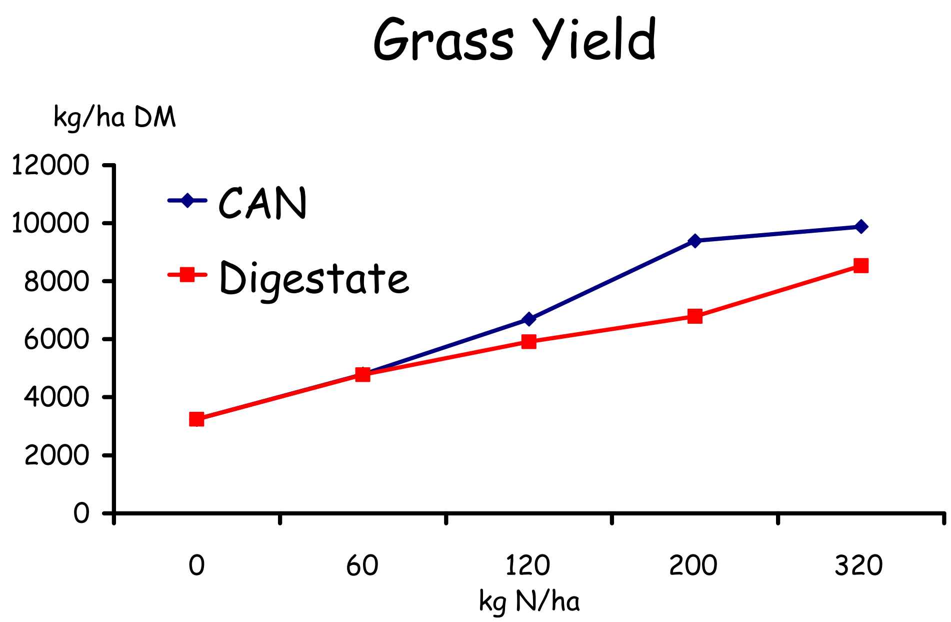


Fig.1. Biomass yield after application of digestate and mineral fertilizer to grass plots at Oak Park

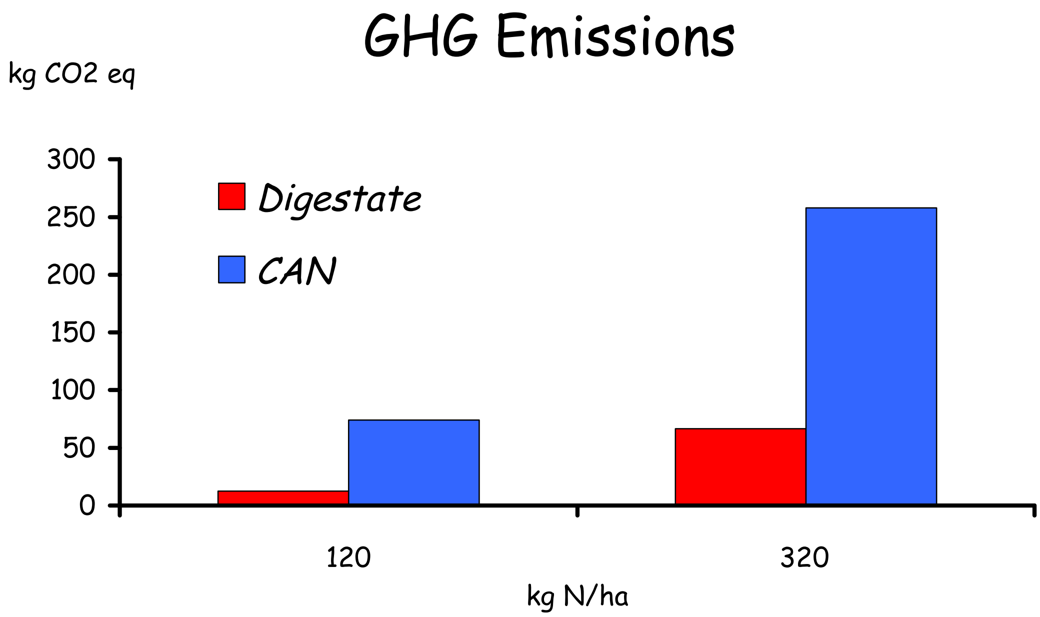


Fig.2. Total emissions of greenhouse gases after application of mineral fertilizer and digestate

Feasability of production and combustion of pellets from straw and energy crops

Finnan, J. and Carroll, J. **RMIS No. 5613**

The use of pellets from sawdust for home heating in Ireland is expanding rapidly. Sawdust has many advantages for pellet production, but its supply is limited and other feedstocks will soon be needed. Short-rotation willow, miscanthus, rape straw and cereal straws are all alternatives of interest to Irish farmers. Pelleting these materials, increases bulk density, which should make them suitable for use in a wide range of biomass boilers and stoves.

During 2009, combustion tests were conducted with pellets manufactured using a range of feedstocks. Combustion was measured in addition to particulate and gaseous emissions and boiler deposits. Results are shown in the table shown.

Table 1: Results of gaseous emissions

	CO (ppm)	NO_x (ppm)	SO_2 (ppm)	Particulates (g/m^3)	Ash in boiler (g/m^2)
Rape Straw	1252.89	241.39	29.76	0.9741	37.87
Miscanthus	83.47	190.90	27.24	0.2413	11.68
Willow	52.34	160.61	12.49	0.0433	1.07
Wheat Straw	117.29	233.84	59.12	0.2703	6.82
Barley Straw	124.45	239.56	47.39	0.2105	5.27

As can be seen from the table above rape straw had by far the largest amount of particulate emission in the flue gases as well as depositing a large amount of ash residues on the boiler walls. These high values as well as the corresponding very high gaseous emission values indicate that pure rape straw pellets may not be a very good alternative biomass. Willow has the lowest values in each of the categories which would be expected as it is very closely related to wood pellet. Miscanthus, wheat and barley straw tend to have very similar values in all emission categories, suggesting these may be very suitable alternative fuels.

Energy Crops and Bioremediation

Finnan J, Ryan, D, Fröhlich, A and Galbally, P. **RMIS No. 5543**

Restrictions on spreading industrial and municipal waste on food crops presents an opportunity to energy crop growers who can avoid fertilizer costs and perhaps gain an income from the spreading of sewage sludge on their crops. However, waste application introduces the additional risk of nutrient loss and heavy metal contamination. The objective of this work was to monitor nutrient loss and soil and water contamination after waste application to energy crops.

In general, there was a relationship between the quantities of nutrients and heavy metals applied in distillery effluent and the concentrations of nutrients and heavy metals in groundwater. Concentrations of nitrate, phosphorus, cadmium, chromium, copper and zinc in groundwater increased with increasing quantities of distillery effluent, this trend was less obvious when municipal solid waste was applied to plots.

Mean nutrient and heavy metal concentrations in groundwater did not exceed the guide values for nutrient and heavy metals in any blocks with the exception of phosphorus concentrations in the blocks which received 100% distillery effluent.

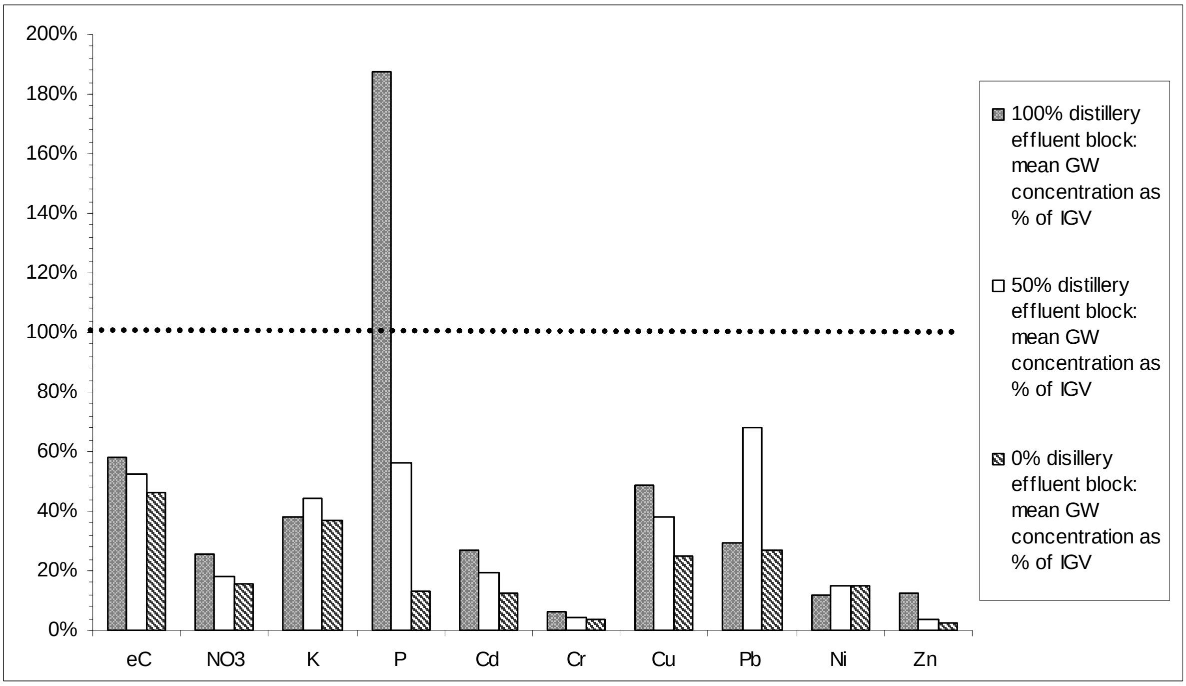


Figure 1 Groundwater concentrations of nutrients and heavy metals in plots which received different concentrations of distillery effluent

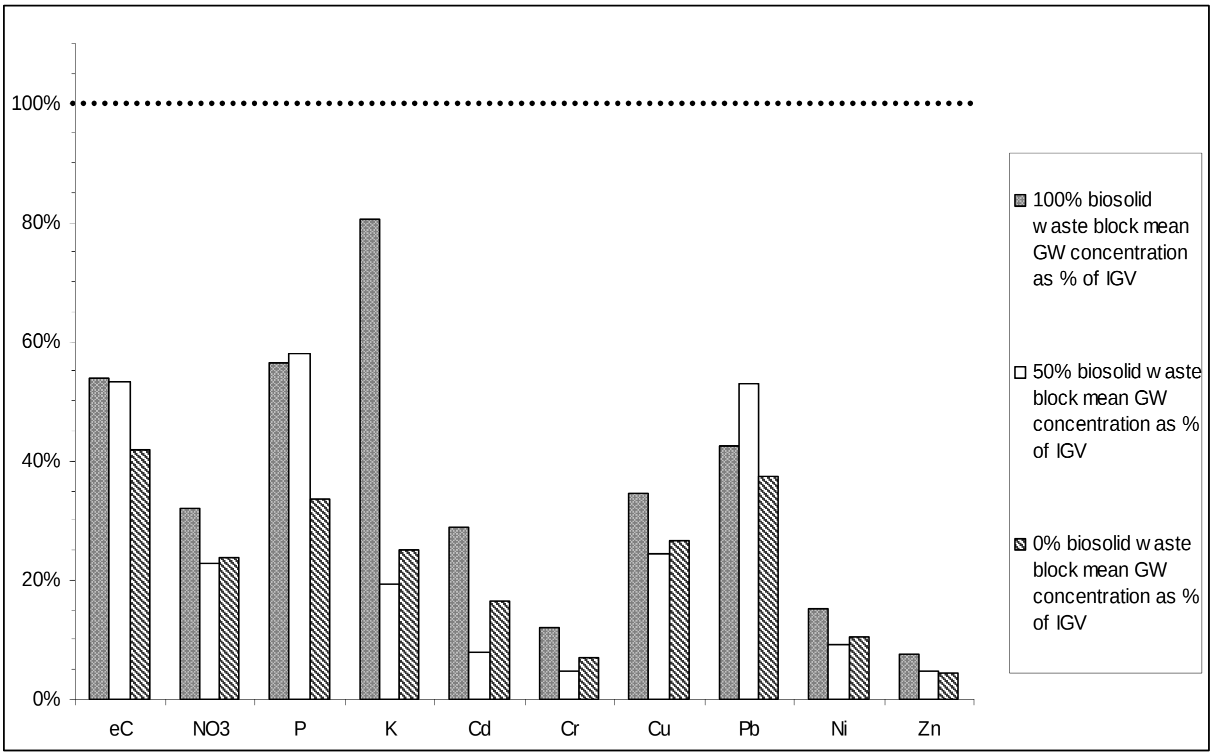


Figure 2 Groundwater concentrations of nutrients and heavy metals in plots which received different concentrations of municipal solid waste

Measurements on surface water or overland flow took place in 2009. Small flow monitors recorded the time, duration and volume of overland flow and took samples for analysis. Concentrations of nitrate, phosphorus, potassium, nickel, zinc and copper in the surface water flowing off plots were proportional to the concentrations of these elements which had been applied to the plots

Biomass Combined Heat and Power

Finnan J, Brett P. **RMIS No. 5915**

Electricity generation typically converts approximately 30% of input energy into output energy. Energy efficiency can be improved significantly if the waste heat from electricity production can be used, a process known as combined heat and power production (CHP). CHP plants can use a variety of feedstocks including biomass. However, small scale biomass CHP plants have not been available until recently and the technology is still maturing. Government renewable energy plans include the production of 800MW of electricity from biomass CHP but this plan will be difficult to achieve unless a range of biomass CHP plant sizes are available.

One biomass CHP technology that is now available is a plant in which an updraft gasifier and gas burner is combined with a stirling engine. A stirling engine is an example of an external combustion engine. In this process heat generated in a boiler is transferred through heat exchangers to the engine where the heat is converted to rotary motion of the stirling engine. The rotational motion of the engine is converted into electricity through an alternator attached to the crankshaft of the stirling engine.

Installation of a biomass CHP plant based on the above principle began at Oak Park during 2009 (see picture below). Wood chips will be augered into the gasifier (black cylinder on the right) and the resulting gas will be burned in the combustion chamber (blue cylinder). Heat from the combustion process will be used to heat water but also to supply electricity through the stirling engine (green). The plant will be used to generate heat and electricity for the research centre but also for research and demonstration purposes.

Figure.1. Biomass CHP plant at Oak Park

Construction of the plant commenced in 2009 when the major components were put in place. The plant is expected to be operational in 2010.

Managing soil organic C in Irish agricultural systems - environmental consequences of land use change for bioenergy.

Burke, J.I, Fitzgerald J. **RMIS: 5675**

This study investigated the changes in soil carbon and N_2O fluxes due to land use change. The study looked at the role of *Miscanthus* in national agri-environmental policy. It evaluated the consequences of land use change for *Miscanthus* using a green house gas (GHG) focussed life cycle analysis (LCA) approach. As *Miscanthus* is a potential fuel source for cofiring at the three Midlands peat-fired powered stations, the area of *Miscanthus* required to fuel 30% cofiring over the course of one year was a focus of the study. It was assumed that this *Miscanthus* was planted on farms where the previous land use was extensive beef production.

Three scenarios were used for comparison. The first was a business-as-usual scenario. The second evaluated the effects of planting 45,000 hectares of *Miscanthus* with the displaced systems being compensated for by intensification of existing farming systems in Ireland. The third evaluated the effects of planting 45,000 hectares of *Miscanthus,* with the displaced systems being compensated for by land expansion abroad.

The study found that scenario two resulted in an emissions saving of ca. 70,000 t CE annum^{-1} or a 0.36% reduction in national agricultural emissions. Scenario three resulted in a saving of 220,000 t CE annum^{-1} in national emissions or 1.15% of total agricultural emissions. However scenario three caused a net increase in global emissions of 325,000 t CE annum^{-1}.

Most uncertainty resulted from soil Carbon and N_2O fluxes due to land use change. The results showed that it is very important to consider baseline land use and displacement effects when assessing the GHG emissions of *Miscanthus* production and utilisation as carbon leakage can result. Geographical boundaries also had a significant effect on the results.

Breeding and evaluation of improved potato varieties for the domestic and seed export markets

Griffin D, Kennedy C, Hutton F, Dalton E. and Milbourne D. **RMIS No. 5612**

Objectives

The objectives for the breeding program in 2009 were to produce varieties with the following attributes

- Early maturity and high yield potential
- Excellent processing and quality traits
- Smooth bright skin finish
- Resistance to late blight and potato cyst eelworm

Seedling evaluation in Ireland

The size of the program remained similar to previous years. The number of seedlings tested at each generation in Ireland and the trial sites used are outlined below.

Table 1: **Number of seedlings under field evaluation in Ireland 2009**

Year of generation	**Type of trial and location**	**No. seedlings under evaluation**
Year 3	Singles in Wicklow mountains	67997
Year 4	Ten tuber lots in Wicklow Mountains	2603
Year 5	Replicated plots (3 replicates each of 30 plants) [Carlow]	191
Year 6	Replicated trials Maincrop: Carlow Early: Wexford	43 11
Years 7-11	Replicated trials: Maincrop: 2-4 sites (Carlow, Wicklow, Louth and Meath) Early: Wexford	43 11
National List Trials (Dept. of Agriculture, and Food)	Maincrop: 3 sites (Cork, Meath, and Kildare)	4

Table 2: Number of seedlings under field evaluation abroad 2009

Country	Partner	No of trials	Type of trial	Location	No of seedlings
United Kingdom	NIAB, Cambridge	1	Clonal	Lincolnshire	31
Morocco	Dynagri	2	Clonal	Soalem and Ziane	74
Spain	PEP Ltd	3	Clonal	Tenerife Seville and Valladollid	71

Trial analysis

All seedlings from years 7-11 are included in the advanced trial series at five sites in Ireland and the UK (northern series) and five sites in Southern Europe/North Africa (southern series). The northern and southern trial series were treated as distinct experiments for analysis purposes. Although different seedlings were included in each experiment a core group of seedlings that were either in national list trials or under consideration for entry to NL trials were included at all sites so a balanced data set could be achieved for the purposes of cross trials analysis. Selected results for advanced seedlings in both trial series are shown below

Northern trial series results:

The yield data was subjected to analysis across sites using the additive main effects and multiplicative interaction (AMMI) model for a 2-way genotype by environment balanced set of data as outlined by Zobel, Wright, and Gauch (1988). Three trials were analysed from sites in Carlow, Waterford and Lincolnshire to achieve a balanced data set. The AMMI ANOVA indicated highly significant differences (P<.001) for genotypes, environments and genotype x environment interaction.

In the AMMI model principal component analysis is conducted on the covariance matrix. In this case the model calculated four principal component axes of which only the first two were significant. A plot of adjusted average yield of the sites and genotypes across the three sites against the first PCA axes scores are shown in Fig 2. The X axis represents the main effects of yield for genotypes and environment while multiplicative interactions are represented by the Y axis. In interpreting this graph, if a variety is very close to zero on the Y axis then it has small interaction effects. When a genotype and an environment have the same sign on the PCA axis the interaction is positive; if different the interaction is negative.

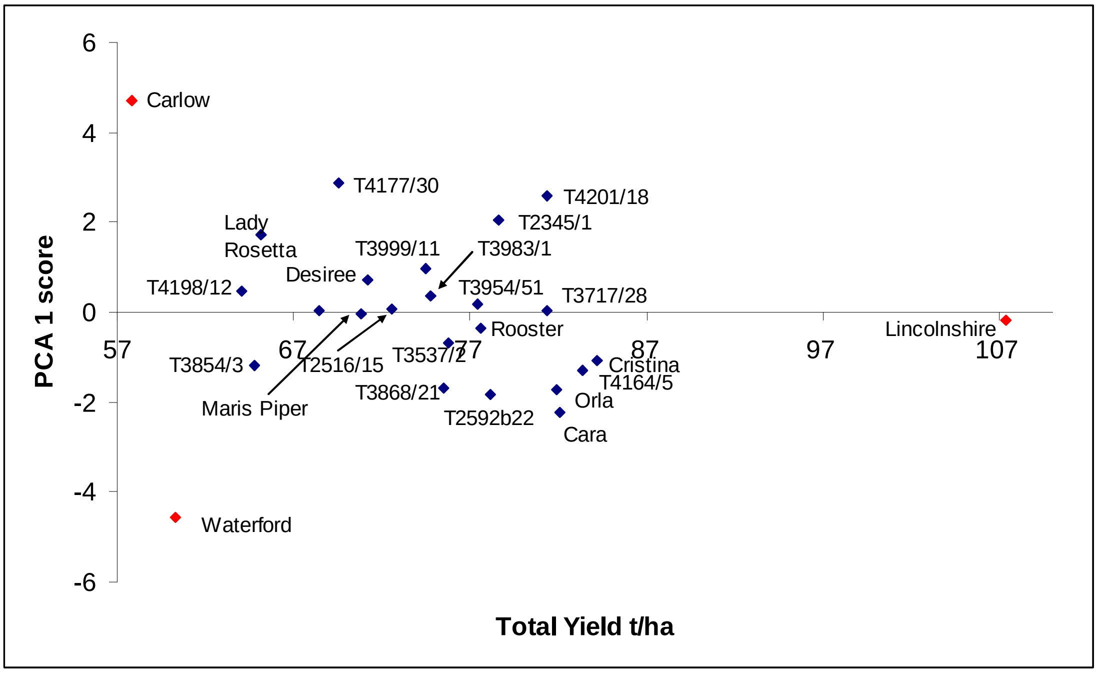


Fig.1. Bi-plot with X axis plotting trial and genotype mean yields (t/ha) and Y axis plotting PCA 1 scores for Northern trial series. Trial means are shown in red and genotype means in blue. Selected controls and advanced seedlings are labelled.

The Lincolnshire site was extremely high yielding in 2009 and considerably higher than the two Irish sites possibly due to the late planting and poor growing season encountered during the summer of 2009. Cristina was the highest yielding seedling in the trial series followed by T4164/5 and Orla. T2516/15, T3983/1 and T3868/21 gave moderate yields but all were considerably higher yielding than Maris Piper and were extremely stable across environments. Lady Rosetta was the lowest yielding control variety in the series. T4201/18 is a crisping variety with similar attributes to Lady Rosetta but was considerably higher yielding in this trial series.

Seedlings which exhibited potential for processing in this trial series included T4198/12 and T4201/18 for crisping and T2516/15 and T3868/21 for French fry production.

Southern trial series results:

Seedling evaluation also took place at five sites in the Mediterranean/North African region in conjunction with Irish Potato marketing and foreign partners.

The yield data was again subjected to analysis across sites using the AMMI model as described previously. The AMMI ANOVA indicated highly significant differences (P<.001) for genotypes, environments and genotype x environment interaction. Results for total yield are shown in Fig 2 below.

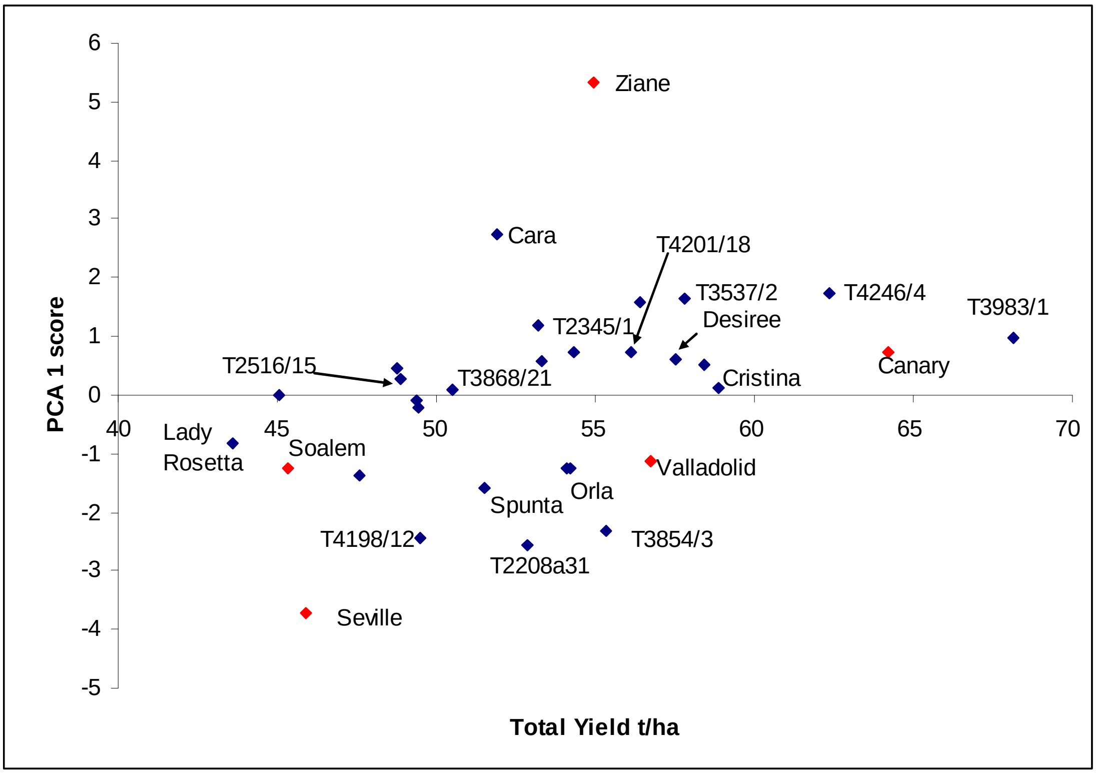


Fig. 2. Bi-plot with X axis plotting trial and genotype mean yields (t/ha) and Y axis plotting PCA 1 scores. Trial means are shown in red and genotype means in blue. Selected controls and advanced seedlings are labelled.

The highest yielding seedlings in this trial series were T3983/1, T4246/4 and Cristina. T3983/1 has been the highest yielding seedling in this trial series for the last three years. Cara however was variable across the sites again showing a positive interaction with the Canaries site. Cara showed a large negative interaction with the three lower yielding sites Seville, Valladolid and Soalem. Lady Rosetta was the lowest yielding seedling across the trial series; however this is due to the varieties extremely high dry matter which makes it suitable for processing. Two seedlings T4201/18 and T4198/12 have similar qualities to Lady Rosetta but had significantly higher yield in this trial series. T2516/15 and T3868/21 both yielded moderately in the Mediterranean series but were extremely stable across the environments. Yield is not the most important driver of selection of potato varieties as many high dry matter varieties may be low yielding but still suitable for industrial processing. Total yield when plotted like this in the AMMI model is a very good predictor of adaptation and stability of performance across different environments.

National List and DUS Trials

Seedlings T3537/2 and T2516/15 completed national list (NL) trials in 2009 after performing well in NL1 trials 2008. Seedlings T3983/1, and T3868/21 completed NL 1 trials in 2009 and T2345/1 was proposed for entry to national list trials in 2010, these advanced seedlings are described below.

T2516/15 is a maincrop variety with white skin. Skin finish is excellent and tuber shape is long oval. The variety has moderate tuber size but has high dry matter and excellent eating quality. It may be suitable for french fry production.

T3537/2 is a deep red skinned round variety with maincrop maturity. Skin finish is excellent. The variety has moderate dry mater levels and a deep yellow flesh. Yield is extremely high and the variety is highly resistant to a range of diseases including late blight.

T3983/1 is a deep red skinned white fleshed early maincrop variety. It is very high yielding and performed well in all southern European sites. Tuber shape and uniformity are excellent. Skin finish may be a little dull. The seedling is particularly suited to North African markets

T3868/21 is a yellow skinned yellow fleshed early maincrop variety. It has potential for both the home ware and export market and is suitable for french fry production. It performed well in both southern and northern European trials.

T2345/1 is a high yielding maincrop seedling. The skin colour is a mix of red and yellow in defined patches which is extremely attractive. The seedling exhibits good disease resistance and similar agronomic characteristics to Rooster. Eating quality is excellent and the seedling is suitable for the Irish market. The skin colour may prove a unique selling point.

Grants of Plant Breeders rights

Varieties Infinity (T3302/3) and Cristina (T3747/13) both received grants of Irish plant breeders rights (PBR) in 2009 and applications have been filed for European PBR which should be granted in 2010.

Infinity is an early main crop deep red variety. Flesh colour is light yellow. The variety is low to moderate yielding but has extremely high dry matter and good fry colours. The variety is suitable for the production of crisps and was consistently higher yielding than the control Lady Claire which is the commercial standard. The variety gives similar fry colours and is showing considerable promise in commercial crisping trials.

Cristina is a high yielding early maincrop variety. Skin finish is excellent and the variety has a very deep red skin with white flesh. The seedling is suitable for prepacking for fresh consumption. It is showing particular promise for UK and North African markets.

The production of virus tested seed stocks of new potato seedlings

The objective of this programme is to ensure that an adequate quantity of virus tested seed is available for multiplication under the seed certification scheme when a new potato variety is recommended for release. It is also designed to produce high quality seed for the commercial evaluation of seedlings at home and abroad.

The production of virus-tested stocks of new potato seedlings was continued at the isolation centres in Wicklow and Myshall during 2009. This covered an area of approximately 6.5 ha and was made up of plots varying in size from single-plant plots to 1,500-plant plots. Virus testing and seed distribution was similar to previous years.

During the winter, samples of all clones are tested for virus infection using the ELISA technique. All infected clones were discarded. During the growing season a total of 59,110 plants representing 777 seedlings were virus tested. This material was subjected to 9,328 serological tests using the ELISA technique. Visual examinations were also carried out at weekly intervals. A total of 95 seedlings were infected with PVY, 3 seedlings infected with PVS, 5 seedlings infected with PVA, 11 seedlings infected with PVX infection with the aphid transmitted virus.

This represents a significant decrease in infection in comparison to previous years. All infected clones were discarded from the propagation programme.

It can be concluded from these results, that with the possible exception of PVY, all advanced seedlings show moderate to high levels of Virus resistance and this should give these seedlings a distinct commercial advantage.

The produce of selected seedlings were sent for trial to 8 countries while the remainder were used for further propagation, disease resistance screening and demonstration.

Meristem-tip culture and micro-propagation

During 2009 meristem-tip cultures of 2 advanced seedlings (T3868/21 + T3983/1) were handed over to the Department of Agriculture and Food for micro-propagation under the Irish Seed Potato Certification Scheme. Following confirmation of the health status, this material is also forwarded to Scotland for multiplication under the Scottish Seed Certification Scheme.

Marker Aided selection

One of the major goals of the potato breeding program is to produce varieties resistant to potato cyst nematode and particularly to the *Globodera pallida* strain. For the last four years markers have been used at the year 4 stage of the program to identify seedlings bred from resistant crosses which have inherited the resistance trait. Screening large numbers of seedling for this trait conventionally is expensive and laborious. Over 400 seedlings were screened in 2009. Seedlings can be positively selected for this trait and if successful in the further two generations of the program the resistance is confirmed using a conventional pot test. Use of marker aided selection has resulted in increased selection of *G. pallida* resistant seedlings at the advanced stage of the program and shows promise for the future.

During 2009 a small scale experimental breeding programme was initiated to investigate the potential for the routine deployment of marker-assisted selection (MAS) as part of the potato breeding programme at Oak Park. In this scheme, MAS is being used to allow the use of recurrent selection in order to rapidly pyramid and multiplex multiple traits into single genotypes over three successive annual rounds of crossing. Traits and resistance sources include resistance to *G. pallida* as mentioned above and also resistance to late blight (*Phytophthora infestans*), *G. rostochiensis* and Potato Virus Y (PVY). A limited amount of phenotypic selection for breeder’s preference is also being implemented. At the end of the three year period, suitably high performing material will be advanced to the main breeding programme.

Disease resistance in new potato varieties

Disease resistance is an important objective of the potato-breeding programme and all advanced seedlings are tested for resistance to a wide range of commonly occurring diseases. Using standard laboratory and field techniques, testing commences when seedlings have completed their seventh year of propagation, and continues until a seedling is discarded or released as a new variety. During 2009 seedlings were tested for resistance to wart disease (*Synchytrium endobioticum*), foliage and tuber blight (*Phytophthora infestans*), common scab (*Streptomyces scabies*), dry-rot (*Fusarium caeruleum and F. sulphureum*), gangrene *(Phoma exigua var. foveata*), Rhizoctonia (*Rhizoctonia solani*), powdery scab (*Spongospora subterranea*), potato virus X (PVX) and potato virus Y (PVY). Many of the new seedlings were found to possess high levels of disease resistance and could make a significant contribution to reducing disease losses and decreasing pesticide use in potato production.

During 2009, 29 seedlings were tested for field resistance to wart disease (*S. endobioticum*) and 5 were found to be susceptible. Seven advanced seedlings were also subjected to laboratory tests for resistance to wart disease. Of these, 1 exhibited the RG1 reaction and 2 exhibited the RG2 reaction while 2 seedlings were considered susceptible.

Testing for resistance to foliage blight confirmed that the most resistant seedlings in 2009 were T3537/2, T4713/9, and T4824/19; however, all seedlings tested were more susceptible than the European standard resistant cultivars, Cara and Robijn.

The seedlings T3999/11, T3868/21, T3954/51, T2516/15, T3302/3, T3537/2 and T3747/13 were highly resistant to dry rot but the remaining seedlings were all susceptible to highly susceptible. T2345/1, T3302/3 and T3537/2 were the 3 seedlings most resistant to scab. Gangrene levels were particularly high in seed crops and advanced seedlings in 2009 and T2516/15 and T3983/1 were most resistant.

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