final report

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Forewarning of ARGT risk for producers

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Abstract

Annual ryegrass toxicity (ARGT) is an acute and often fatal neurological disease caused by consumption of annual ryegrass (*Lolium rigidum*) seed-heads infected with corynetoxins produced by a bacterium, *Rathayibacter toxicus*. It is a problem in southern Australia and in particular in the south west of Western Australia where *R. toxicus* now is endemic. There is essentially a nil tolerance of contamination of Australian export cereal hays with *R. toxicus*. Since 2005 testing of hays destined for export has been mandatory and now is governed by AQIS requirements. The fodder industry in Australia is worth in excess of $1billion *pa*. It supplies the dairy, beef, horse and sheep industries domestically, and the export of fodder hays is large and increasing. Although the risk of deaths of grazing and lot-fed livestock due to ARGT can be high, these risks have not been as well documented as those in the export cereal hay industry.

The outcome of this study is a web-page on the website of the Department of Agriculture and Food Western Australia where the potential risk of incidence of the causative agent of ARGT, *R. toxicus*, is mapped each year across the agricultural region of Western Australia by July 30. This provides producers of cereal hays fore-warning of the risk of incidence of ARGT each year, and can alert producers of cereal grains and of grazing livestock in the same areas to be aware of the risk of ARGT. Producers can then take appropriate action.

Executive summary

The summary of this study is what now is provided on the DAFWA website, the principal objective of this study (below).
Go to http://www.agric.wa.gov.au/cropdisease and follow link for ARGT risk forecast 2011.
The website appears as follows:

ARGT risk forecast 2011

ARGT is a disease of livestock that results from the consumption of sufficient quantities of toxic ryegrass seedheads. The seed heads are rendered toxic when they are infected by a bacterium, *Rathayibacter toxicus*, which is introduced into the seedhead by a nematode, *Anguina funesta*. The disease can arise from the consumption of any feed that contains the toxic seedheads, including pasture, crop stubbles, hay and grain.

For ARGT to occur the toxic bacterium must be present. When it is present the risk of the bacterium building up to levels that are dangerous is principally influenced by weather patterns before and during the growing season. These patterns include false breaks, the incidence and intensity of early rainfall events and temperature patterns. However, the risk can be modified by implementation of pasture and grazing management practices that minimise the production of the toxins that cause ARGT, or by use of the resistant ryegrass ‘Safeguard’ or the biological control agent twist fungus (*Dilophospora alopecuri*). There are naturally-occurring populations of twist fungus in some parts of the SW Agricultural region. Conversely, the risk may be increased unintentionally by, for example, causing a pseudo false break as a result of an effective knockdown prior to seeding followed by a second germination event.

More information can be found on the DAFWA website at ARGT.

Here, potential risk, assuming the presence of *Rathayibacter toxicus* and a few mitigating factors, of the incidence of ARGT is predicted using daily weather data from weather stations across the SW Agricultural region each year until July 30. Risk has been predicted as the proportion of properties tested exhibiting ARGT, it does not include an indication of the intensity of *R. toxicus* on individual properties. Where, all of the precursor elements required for an outbreak of ARGT are present, without any mitigating management practices then the risk prediction will assist producers to assess the management options of their hay crops or pasture, and livestock, to manage the risks of ARGT.

Where there is a history of ARGT on a property or in a paddock the predictions here must be treated with caution depending upon management actions that may have unintentionally exacerbated the possible development of ARGT.

Equations used to estimate relative risk were developed from information from the export hay industry in the years 2000 to 2005. In the maps relative risks are expressed in categories of 0% (pale green) to 100% (dark green) in intervals of 20%. The relative risk is an indicator that management decisions may need to be taken (see above).

Although the hay industry is concerned primarily with levels of the bacterium well below those required to cause clinical disease (the export hay industry works on a virtual nil tolerance for presence of the bacterium), it is expected that the predictions will be equally applicable to farmers considering the risk of ARGT in their pastures, crop stubbles and meadow hay as to export hay producers.

For further information contact Dr Jeremy Allen.

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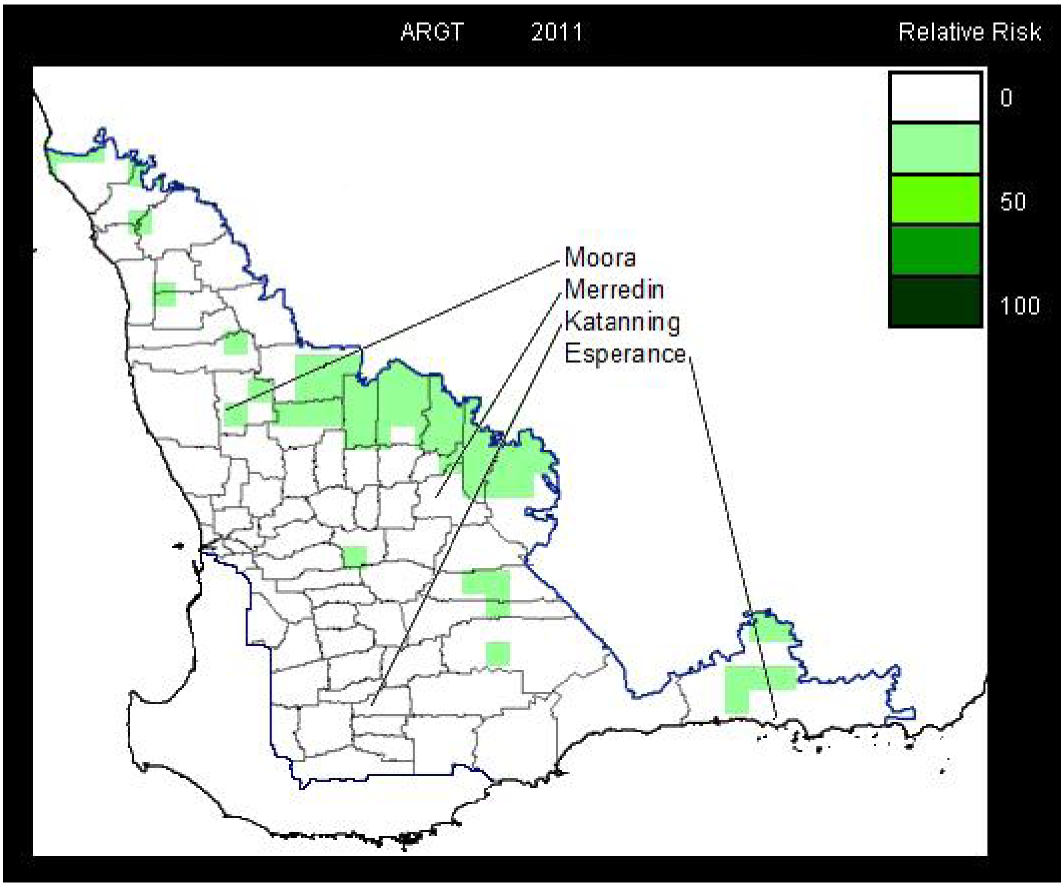

In this study, a substantial database was used. It was established in a previous RIRDC-funded project (BSC-1A, Baker and Purser, 2008) and in the 52,561 data fields that were recorded were incidence and severity of *R. toxicus* in cereal hays for export identified by longitude and latitude of growers' paddocks and associated weather information over six years (2000 to 2005). In that data base, estimates of date, time and number of false breaks to the season were included. In this study substantially more data have been added from daily weather data over those six years including estimates of evapotranspiration, soil moisture and an index of greenness. This large amount of data has been used to predict the risk of incidence of *R. toxicus* into the future with a reliability of prediction (R^2) in the base years, 2000 to 2005, of between 0.53 and 0.70. This is a reliable estimate given such a large range of weather conditions in those years and hay-producers' management decisions during the years of the study.

Contents

1 Background

Annual ryegrass toxicity (ARGT) is an acute and often fatal neurological disease caused by consumption of annual ryegrass (*Lolium rigidum*) seed-heads infected with corynetoxins produced by a bacterium, *Rathayibacter toxicus*. There is essentially a nil tolerance of contamination of Australian export cereal hays with *R. toxicus*. Since 2005 testing of hays destined for export has been mandatory, and now is governed by AQIS requirements (AQIS, 2005). The fodder industry in Australia is worth in excess of $1billion *pa*. It supplies the dairy, beef, horse and sheep industries domestically, and the export of fodder hays is large and increasing (AFIA, 2009). Although the risk of deaths of grazing and lot-fed livestock due to ARGT can be high, these risks have not been as well documented as those in the export cereal hay industry. Results from this study now will provide producers of cereal hays fore-warning of the risk of incidence of ARGT and identify conditions where producers of cereal grains and of grazing livestock in the same areas would need to be aware of the risk of ARGT.

Corynetoxins occur in grasses parasitized by a complex of a nematode (*Anguina funesta*) and the corynetoxin-producing bacterium, *R. toxicus*. Annual ryegrass (*L. rigidum*) is the grass most commonly infested by the nematode-bacterium complex, and annual ryegrass is an important weed in cereal crops of southern Australia. It also is an important pasture species for many livestock producers. The biology of the nematode-bacterium complex, the parasitism of annual ryegrass and the pathology of corynetoxin poisoning of livestock have been reviewed extensively (see for example, McKay and Ophel (1993) and Finnie (2006)). Briefly, annual ryegrass seedlings are colonized soon after germination in autumn by larvae of *A. funesta*. The larvae are carried up as the plants grow, invading the tillers and then inducing galls in the developing inflorescences, in which the larvae develop into adults and reproduce. The larvae of *A. funesta* are an essential vector for *R. toxicus*, which adhere to the larvae in the soil and multiply in the galls induced by the nematode. Under certain conditions, the bacterium multiplies rapidly in the gall and the nematodes atrophy and die, so that galls will contain predominantly either bacteria or nematodes. Finally, production of corynetoxins by *R. toxicus* appears to be induced by infection of the bacterium by bacteriophage. These events are influenced strongly by weather patterns. Rainfall events in summer and early autumn generally are not sufficient to sustain annual ryegrass through to the reproductive stages of its life cycle but sometimes can be sufficient to initiate seed germination and support early seedling development, after which the seedlings die. *A. funesta* in the galls of seed shed in the previous growing season remain dormant in the soil until the autumn or early winter rains, and the infective larvae emerge between two and six weeks after there has been sufficient soil moisture to soften the walls of the galls. Reproduction rates of the nematodes are greatest in short growing-seasons, and thus in early-maturing ryegrasses. Similarly, if the vegetative phase of the ryegrass is short, more nematode galls are formed than if the season is longer (for example in high-rainfall areas) and/or if the ryegrass is a late-maturing variety.

The stages of development in the lifecycles of the ryegrass, the nematode and the bacterium are important in the development of the corynetoxins by *R. toxicus*, and available evidence indicates strongly that these stages of development depend on seasonal conditions (weather patterns and weather events). The geographic distribution of the causative bacterium, *R. toxicus* in pastures, crops and grains is wide throughout southern Australia (see for example, McKay *et al*., 2000 and Kessell, 2010). The geographic area in which oaten hays are grown in southern Australia is large and weather patterns and events differ across the region within and between years.

In a previous study, (RIRDC project BSC-1A; Baker and Purser, 2008) analysis of weather patterns confirmed that an increased risk of the presence of R*. toxicus* and thus risk of ARGT is likely to be associated with a late start to the season and/or weather conditions leading to early maturity of plants in spring (low rainfall and high temperatures). In very general terms, this can be interpreted that as the number of false breaks to the plant growing season increases the risk of ARGT increases and this risk is further increased by a short growing season. It is possible, given what is known of the biology of *R.toxicus*, *A. funesta* and annual ryegrass germination, that at each false break some nematode galls break down and release their nematode larvae into the soil. With the warm temperature and subsequent drying the nematodes go into hibernation in the soil, and with each break there is an increased number of hibernating nematodes in the soil. When the true break of season comes, with the cooler conditions there is a large bank of nematode larvae immediately available to actively seek out newly germinated ryegrass, resulting in many more ryegrass plants being colonised. This suggests that there is likely a relationship between predicted soil moisture and the risk of ARGT. The results from BSC-1A provide a template that was tested more fully in this study, using a larger data set and further estimations from the weather data as predictors of incidence of *R. toxicus* and risk of ARGT.

2 Project objectives

The objective of this study was to provide advance warning for cereal hay, cereal grain and livestock producers of weather patterns that are likely to increase the potential risk of incidence *R. toxicus* and in turn ARGT. This would be achieved by making predictions of potential risk of ARGT available on the web-site of DAFWA. Prediction would be made using weather information, and would be based on the outcomes of a previous RIRDC-funded project (BSC-1A, Baker and Purser, 2008) and this study. Weather data to July 1 would be used so that a prediction of potential risk would be available early in the growing season to allow producers to take corrective action if it they wish.

3 Methods

In this study, the substantial database established in a previous RIRDC-funded project (BSC-1A, Baker and Purser, 2008) comprising 52,561 data fields in which incidence and severity of *R.toxicus* in cereal hays for export were identified by longitude and latitude of growers' paddocks and associated daily weather information over six years (2000 to 2005). This database represented *ca*. 44% of samples of hay for export tested by DAFWA for presence of *R. toxicus* in that period of time. In BSC-1A, a sub-sample of the database was used and the weather conditions that were the most significant predictors of incidence of *R. toxicus* were:

- Number of false breaks to the plant growing season
- Average daily maximum temperature for September
- Average daily rainfall for September
- Average daily potential evapotranspiration for September.

In BSC-1A only *ca*. 40% of the database was used. In this study, the entire database was used. In addition, further potential predictors of risk of ARGT were added. Importantly, in this study, weather variables and estimates made from them were only from January 1 to July 1 in each year. This shorter time frame was chosen because it would enable producers to take action to manage ryegrass in pastures in the current year. Better predictions can be obtained by using variables to September or October but it is of lesser use as a forewarning system.

The additional potential predictors that were developed were:

- Estimated greenness indices based on a model developed by DAFWA. (http://www.agric.wa.gov.au/content/PW/PH/DIS/GREEN_BRIDGE.HTM) From that web-site the greenness index is explained as follows:
  'The greenness model utilises daily temperature, rainfall and evaporation data to determine the greenness level or build up and break down of green bridge plant material. This can include growth of weeds, crop volunteers, pasture, etc. both before and during the growing season in south western WA. Temperature determines the rate at which greenness increases. Rainfall and evaporation are used to determine the fraction of plant available water, which is then used to calculate the maximum capacity for greenness. The model outputs have been calibrated using satellite NDVI (Normalised Difference Vegetation Index) imagery.'
- Estimated soil moisture, using two algorithms (discussed later).
- Weather variables estimated at half-monthly intervals, rather than at the monthly intervals used in BSC-1A.

Greenness index and estimated soil moisture possibly relate to the relationships between break of season, false breaks and the biology of the bacterium, the nematode and the germination of ryegrass. Weather variables at half-monthly intervals were used to provide a finer focus on the potential movement of the nematode through the soil, as it is two to six weeks before nematode-containing galls break down and the nematodes emerge into moist soil (see review by Finnie, 2006).

All the data in the database are spatially (geographically) explicit, that is they are not constrained by Shire boundaries. The weather variables came initially from SILO 'data-drill', an interpolation by longitude and latitude with a resolution of 2.5km. The weather data used finally in this study and now on the DAFWA website for prediction of risk of incidence of *R. toxicus* are uploaded daily to DAFWA from 188 weather stations across the south west of WA, and interpolated using patched point data (PPD) algorithms which have a resolution of 4km. Unfortunately the weather data currently available to DAFWA were not the same as the range of weather data provided by the SILO 'data drill'. Thus there were two tasks: the first to find correspondence between predictions using the two available ranges of weather data and the second to find correspondence between the two spatial resolutions. Comparisons were made by simple linear regression.

To make the predictions, the spatially (geographically) explicit estimates of incidence of *R. toxicus* were related to a measure of relative risk (RR). RR of incidence of *R. toxicus* was estimated using SaTScan (Kulldorff, 2006), software developed by New York Department of Health to trace and predict human-health incidences and used widely throughout the world. In SaTScan expected incidence of disease is modelled using a Monte Carlo procedure for each geographic location. RR is calculated as observed incidence of *R. toxicus* compared with expected incidence, and expressed as a percentage (%), or alternatively as observed incidence/total observations; both give the same result (discussed later).

RR(%) of incidence of *R. toxicus* was subjected to multivariate analyses using partial least squares regression (PLSR) in a software program, UnScrambler® (Version 10.01, Camo Software, Norway). In this analysis there were 89 variables used for each year in the retrospective analysis (years 2000 to 2005) in order to make prospective analyses: the half-monthly data for each weather variable, from January to July, estimates of season break (day of break, number of false breaks, rainfall in each break and break relative to the long term average), estimated evapotranspiration, estimated soil moisture and estimated greenness index (see later). These were used to develop prediction equations of the incidence of *R. toxicus*, which then were mapped using the DAFWA PPD weather station data. These maps now will be available each year on the DAFWA website.

- **Evaluation of predictors of estimated greenness index and estimated soil moisture**

Two estimates of soil moisture were made, and compared with estimates of using the greenness indicies for three sites for the period 2004 to 2006. These analyses were made by Moin Salam and Tim Maling of DAFWA.

a) A greenness index was estimated using daily data and integrated for half-monthly periods from the beginning of each month in each year. This was included in the database to estimate the potential for a 'greenness bridge' for transfer of the nematode (*A. funesta*) and the bacterium (*R toxicus*) through the soil.

b) Two estimates of soil moisture were considered. They were included in the database. These estimates were calculated from daily weather data and were integrated for half-monthly periods from the beginning of each month for each year. Estimates of soil moisture were made with two estimators of potential evapotranspiration because in the same retrospective period (2000 to 2005) the same data were not available in the patched-point data (PPD) and SILO 'data drill' data (the latter was used in the previous project BSC1A). That is, PPD data were used in this study for prediction, and it has a coarser geographical resolution that the SILO 'data-drill' data. The predictors of likelihood of incidence of *R. toxicus*, the causative agent of ARGT, in the previous project (BSC-1A) were: false breaks to the plant growing season, and the rainfall, temperature and potential evapotranspiration late in the plant growing season. This re-analysis was undertaken because soil moisture seems to be important in the migration of the nematode (*A. funesta*) to germinating seedlings of annual ryegrass (and other host plant species).

   Two algorithms to estimate soil moisture finally were selected: one using constants for pan evaporation and a crop, K_p and K_c respectively, to estimate potential evapotranspiration, ET_0, and the other using FAO56 to estimate ET_0. FAO56 is a method to estimate evapotranspiration, ET_0, of a crop based on the Penman-Monteith method (Allen *et al*, 1998). The FAO56 estimate of ET_0 is available from SILO 'data-drill', and was a significant predictor of incidence of *R. toxicus* in the previous project (BSC-1A). It is not yet available in the available PPD weather variables. K_p and K_c were calibrated with a decision support software, APSIM (Agricultural Production Systems sIMulator, McCown *et al*., 1996. http://www.apsim.info), using a soil depth of 30cm and a maximum water-holding capacity of the soil of 60mm, as it was considered unlikely that 'germinating' nematode galls and the incidence of the causative organism of ARGT, *R. toxicus*, would be found at deeper soil depths. The crop used in the APSIM simulation was oats, because the APSIM model does not have a ryegrass module.

   The correlation between the two algorithms, 'new' model and FAO56, for estimation of soil moisture at three sites, Merredin, New Norcia and York, yielded

R^2 values of 0.966, 0.996, and 0.993 respectively (Table 1). These algorithms then were used to calculate estimates of soil moisture for *ca*. 1300 sites in south western Australia using the SILO 'data drill' daily weather information for the years 2000 to 2005 . The data used were potential evaporation (FAO56, Allen *et al* 1998), pan evaporation, and rainfall.
The 'new' model of evapotranspiration from this analysis used estimated pan evaporation from the PPD, a crop constant, and a pan evaporation constant to estimate evapotranspiration (Et_0) given the formula $ET_0=E_p*K_p*K_c$

Where ET_0= simulated evapotranspiration (mm)
E_p= pan evaporation (mm)
K_p= pan evaporation constant=0.8
K_c= crop constant= 0.95

The soil moisture model used evapotranspiration and rainfall to estimate soil moisture given the formula:

$Sm=Sm(day-1) + Rf – ET_o$

Where Sm=soil moisture (mm)
Rf=rainfall (mm)
ET_0= evapotranspiration (mm) (either FAO56 or 'new' model ET_0)
And, Sm (max)= 60 (mm)

Comparisons of simulated soil moisture using either FAO56 evapotranspiration data, or the 'new' model to estimate Et_0 at three sites were compared, Merredin, New Norcia, and York for the years 2004-20066 and are shown in Figures 1 to 6.

The values used in Figures 1 to 6 were evaluated such that ET_0 and soil moisture values using 'new' model (x) were regressed upon ET_0 or soil moisture values using FAO56 (y) for each site (Table 1).

Regression of the 'new' model ET_0 estimations on FAO56 ET_0 estimations gave R^2 values of 0.919, 0.799, and 0.912 for Merredin, New Norcia and York respectively (Table 1). The poorest correlation (0.799, at the New Norcia site) was a site where at which the 'data drill' interpolated weather data location was furthest from the nearest PPD weather station. In the Figures, it seems that the 'new' model ET_0 underestimated evapotranspiration during the summer months compared with FAO56 ET_0, but they agree during the growing season. Given that soil moisture during summer is negligible it should not have much effect on predicted soil moisture.

Indeed, regression of soil moisture estimations derived using the 'new' model ET_0 and those derived using FAO56 data gave R^2 values of 0.966, 0.996, and 0.993 for Merredin, New Norcia, and York respectively (Table 1). This provides confidence that whilst the underestimation of ET_0 with the 'new' model in the summer months decreases direct ET_0 to ET_0 correlations, this effect is mitigated by the lack of soil moisture in those months, providing tight correlations between soil moisture estimates using both derivations of ET_0.

The comparisons of these two estimates of soil moisture with the APSIM simulation are useful, but of limited value as APSIM requires more detailed soil information than was possible in this study. Finally, the greenness index was compared graphically with the estimated soil moisture at the three sites, Merredin, New Norcia and York using the 'new' model (Figures 1 to 6). The patterns of greenness index and soil moisture for each of those years (2004 to 2006) are what accord with local experience, as was the case for false breaks in BSC-1A.

The conclusion from these analyses was that ET_0, estimated soil moisture using both the 'new' model and using FAO56, as well as the greenness index were included in the database used in this study.

Table 1. Summary of regression comparisons of two models of evapotranspiration (the 'new' model and FAO56) and estimated soil moisture (the 'new' model and FAO56) from Figures 1 to 6. In this analysis n = 1096, and p = 0.00.

	Estimated evapotranspiration (ET_0) from the 'new' model compared with FAO56 .		Estimated soil moisture (mm) from the 'new' model compared with FAO56 .	
Site	Adjusted R^2	SE[1]	Adjusted R^2	SE[1]
Merredin	0.919	0.6028	0.966	2.7506
New	0.799	0.9375	0.997	1.2124
York	0.912	0.6149	0.993	1.6516

[1] Standard error of the regression

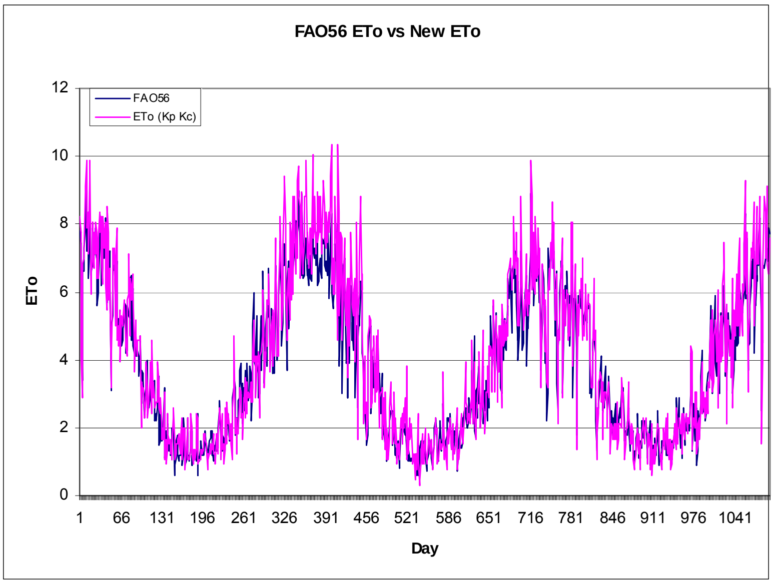

Figure 1. FAO56 ET_0 vs 'new' model ET_0 at Merredin for the years 2004 to 2006, with day expressed as day since January 1 2004.

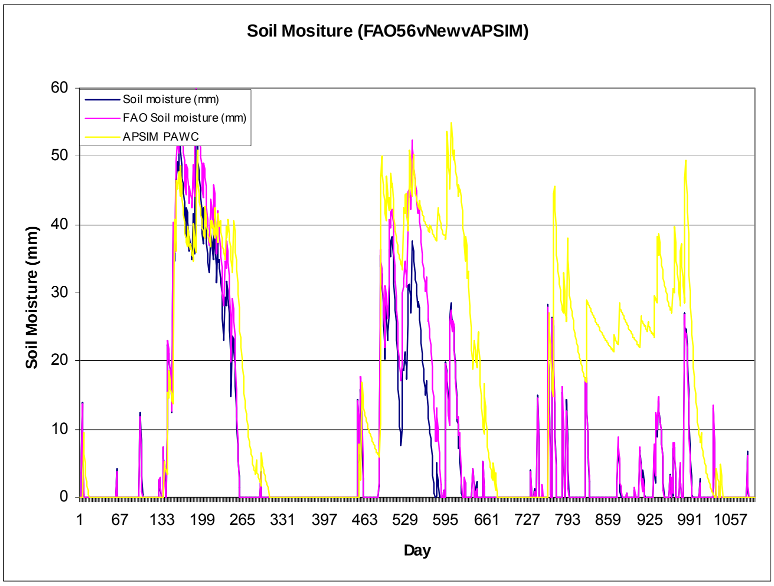

Figure 2. FAO56 ET_0 vs 'new' model ET_0 and APSIM simulation at Merredin for the years 2004 to 2006, with day expressed as day since January 1 2004.

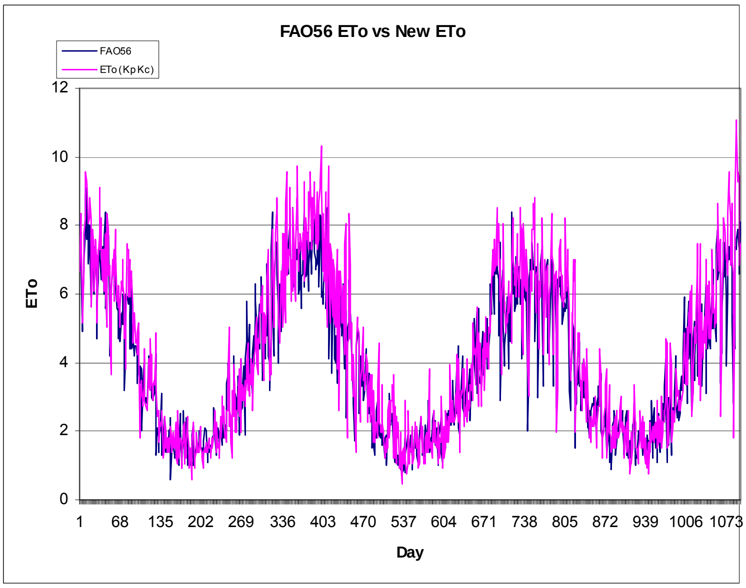

Figure 3. FAO56 ET_0 vs 'new' model ET_0 at New Norcia for the years 2004 to 2006, with day expressed as day since January 1 2004.

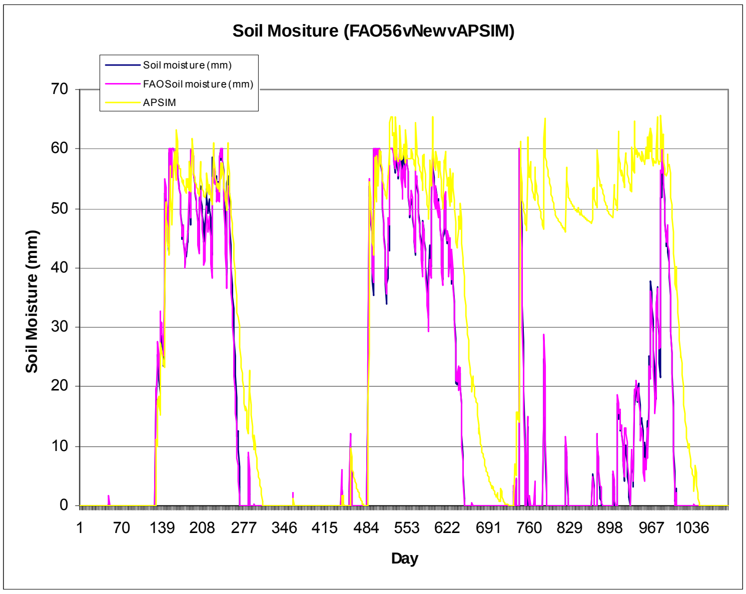

Figure 4. FAO56 ET_0 vs 'new' model ET_0 and APSIM simulation at New Norcia for the years 2004 to 2006, with day expressed as day since January 1 2004.

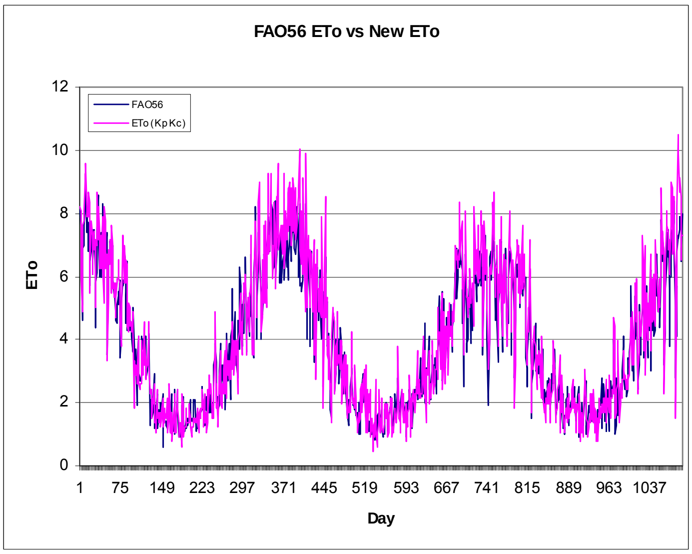

Figure 5. FAO56 ET_0 vs 'new' model ET_0 at York for the years 2004 to 2006, with day expressed as day since January 1 2004.

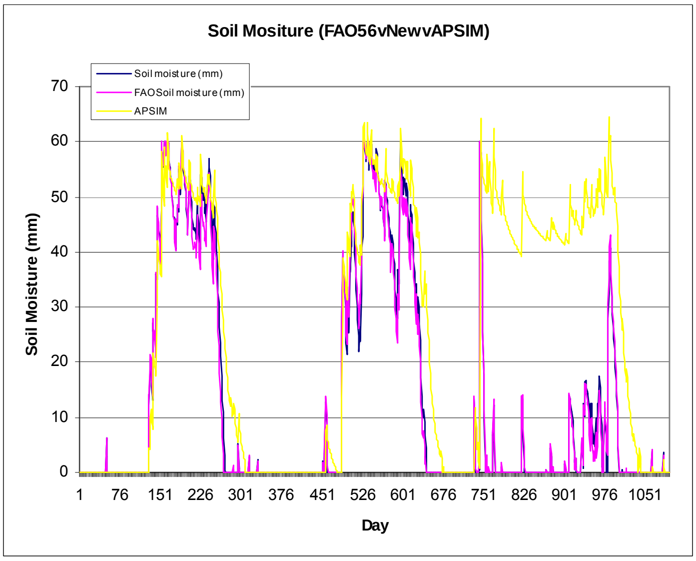

Figure 6. FAO56 ET_0 vs 'new' model ET_0 and APSIM simulation at York for the years 2004 to 2006, with day expressed as day since January 1 2004.

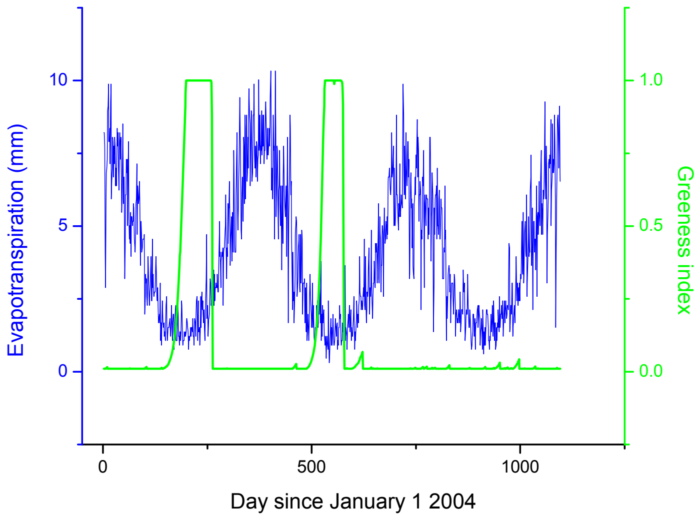

Figure 7. Estimated evapotranspiration (mm), using the 'new' model and greenness indices for Merredin for January 1 2004 to December 31 2006.

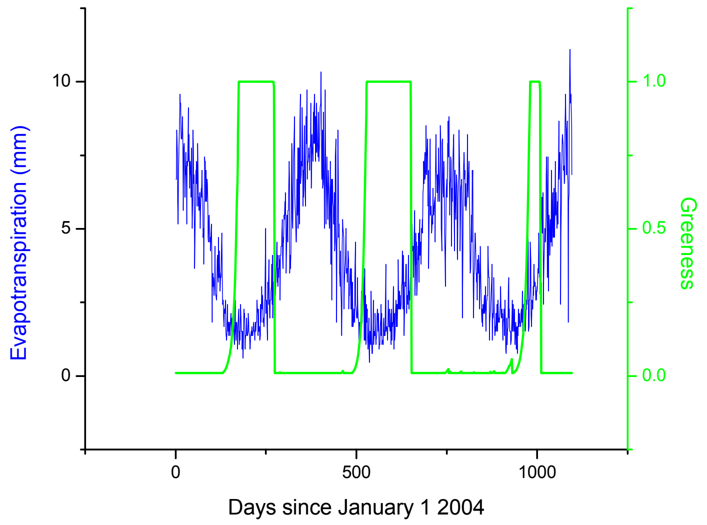

Figure 8. Estimated evapotranspiration (mm) using the 'new' model and greenness indices for New Norcia for January 1 2004 to December 31 2006.

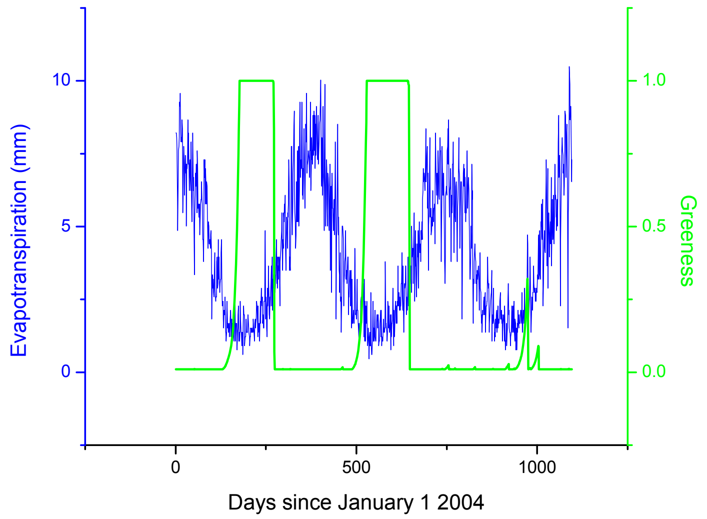

Figure 9. Estimated evapotranspiration (mm) using the 'new' model and greenness indices for York for January 1 2004 to December 31 2006.

- **Translation of the data from SILO 'data drill' to PPD.**

In the original data set (in BSC-1A) with the longitude and latitude co-ordinates of the growers' properties weather data and derived weather variables used interpolated data from SILO 'data drill'. Those data were not available to DAFWA from its PPD sites (see DAFWA Farmnote 111). So, a conversion of those 'data drill' information and the associated growers' data to the spatially (geographically) coarser PPD was necessary. This involved concatenation of the original data into the longitude and latitude coordinates of the DAFWA PPD sites.

- **Discussion of the statistical approaches taken to evaluate the incidence of *R. toxicus* in the restropsepective data set.**

The data in this study of the incidence of *R. toxicus* are continuous data of EIA units (EU) in export hays. It was decided to use these data as count data, classified as samples with < 20EU which are accepted for export and samples with > 20EU which are rejected for export. That is, there are two discrete outcomes from each set of EIA analyses from a paddock (defined by longitude and latitude). This classification of incidence of *R. toxicus* is consistent with medical and veterinary epidemiology – either the disease is present or it is absent, and it is consistent with AQIS requirements for export hays (AQIS, 2005).

This then presented a problem in statistical analysis because the distributions of data in this study was strongly skewed, and most statistical analyses assume a normal distribution. This is common in epidemiological and ecological situations; there are a large number of zero counts, resulting in what is known as an over-dispersed distribution, or a zero-inflated distribution. These distributions are not easily handled to 'stabilize' the variance of the distribution such that the transformed distribution converges to normality. This was discussed in BSC-1A (Baker and Purser, 2008).

These are instances of discrete probability distributions in which there are successes or failures within several independent experiments (n), each of which yields success (a yes or no outcome) with a probability p. A success/failure experiment is also called a Bernoulli experiment. These are a family of probability distributions known as binomial distributions, and when $n = 1$, the binomial distribution is a Bernoulli distribution. Binomial distributions often are used to model the number of successes in a sample of size n from a population of size N, where the population size N is much larger than n. The probability of success (p) = (number of successes)/(sample size (n)). The binomial distribution converges toward a Poisson distribution as the number of trial increases, that is if n is sufficiently large and p sufficiently small. Two rules of thumb are that if $n \geq 20$ and $p \leq 0.05$, or that if $n \geq 100$ and $n*p \leq 10$. If n is large enough, the skew of the distribution is not too great, and a suitable transformation of the data is used, then the binomial distribution converges toward a normal distribution. 'Rules of thumb' are that p should not be near 0 or 1, n should be > 5, and the variance of the transformed distribution should be within 3 standard deviations of the mean (Box *et al*., 2005).

In this study two approaches were taken to normalize the data and stabilize the variance:

a) Truncate the data by deleting the zero values. This is the approach taken in the previous work and was used in this study.
b) Transform the count data by a procedure (FTP) available in 'Minitab® (Minitab Inc. from http://www.minitab.com/en-US/support/answers/answer.aspx?ID=277). It is an arcsine transformation (references cited at that site: Bisgaard *et al.,* 1994; Freeman and Tukey, 1950).

There was no discernable effect on the incidence (probability, p) of risk of ARGT, defined as (count of cases >20EU)/(total count) (p = 0.31, Table 2). Comparison of the two methods of expressing these data also were not statistically different and the FTP transformation was used in a comparison of the data from *ca*. 40% of the database from BSC-1A.

Table 2. Results of comparison of truncated data set and transformed data set.

	Total count (observations)	Count > 20EU (positive count)	*p* (positive count/total count) Also known as 'risk'	*n* (number of 'experiments) ie sample size
Focus group data[1]	25029	7844	0.31	197
Focus group data, using truncated data[1]	24666	7844	0.32	124

[1]Locations from cluster 2 in BSC-1A (Baker and Purser, 2008)

- **Expression of risk of incidence of *R. toxicus***

The approach taken here was to predict risk (probability of incidence) of *R. toxicus* from weather patterns. The prediction of risk from probabilities of incidence is similar in other epidemiological studies. However few, if any, studies have attempted to do this for crop diseases. Most crop disease predictions come from models of biology of disease incidence. However in this study relative risk is expressed as:

Incidence risk = (number of incidences of >20EU)/(sample size (*n*)).

This is similar to RR(%) calculated in SatTScan where the equation is

RR(%) = number of cases identified/number expected, assuming a Poisson distribution in a Monte Carlo analysis (Kulldorf, 2006).

The two expressions of risk were not significantly different for the years 2000 to 2005 ($p < 0.05$). The final analysis of the data in this study was with RR% from SaTScan as the key variable.

Collation of SILO 'data drill' and PPD, weather data and calculations from them, were made by Peter Kovesi who wrote the algorithms in MatLab. The data were summarized with descriptive statistics using Microsoft® Excel and Minitab® (Version 15). Data were plotted and some statistical analyses were made using OriginPro® (Version 8, OriginLab Corporation, USA). The conversion of the output from the Matlab programming language to the Java programming language to be automated and uploaded to the DAFWA website each July was made by Tim Maling.

4 Results and discussion

The principal objective of this study was to provide maps for the agricultural region of Western Australia of the distribution of risk of incidence of *R. toxicus,* the causative organism of the corynetoxins which is responsible for ARGT, on the DAFWA web-site in July each year. This was achieved for the 2011 growing season. It will be ongoing, being provided on the DAFWA website in July each year, using the algorithms established in this study. The distribution of relative risk (RR) of incidence of *R. toxicus* across the agricultural region of south-western Australia is mapped for the years 2000 to 2005 using retrospective data (Figure 10) and predicted for the years 2006 to 2011 (Figure 11). Lines on the map indicate Shire boundaries. These predictions are spatially (geographically) independent of Shire boundaries.

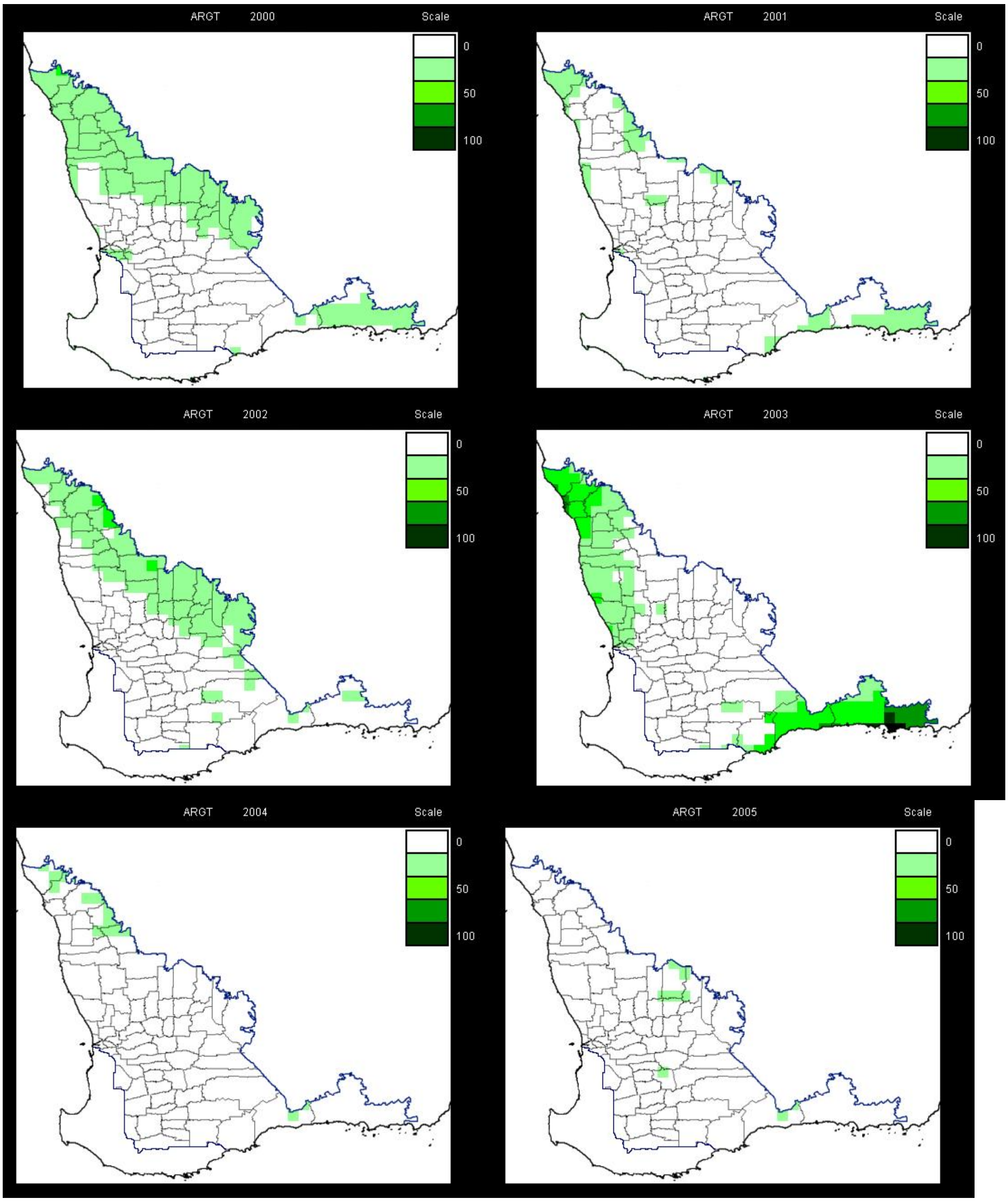

Figure 10. Distribution of relative risk of incidence of *R. toxicus* in the agricultural region of south western Australia for the years 2000 to 2005, the years upon which the data analyses were made.

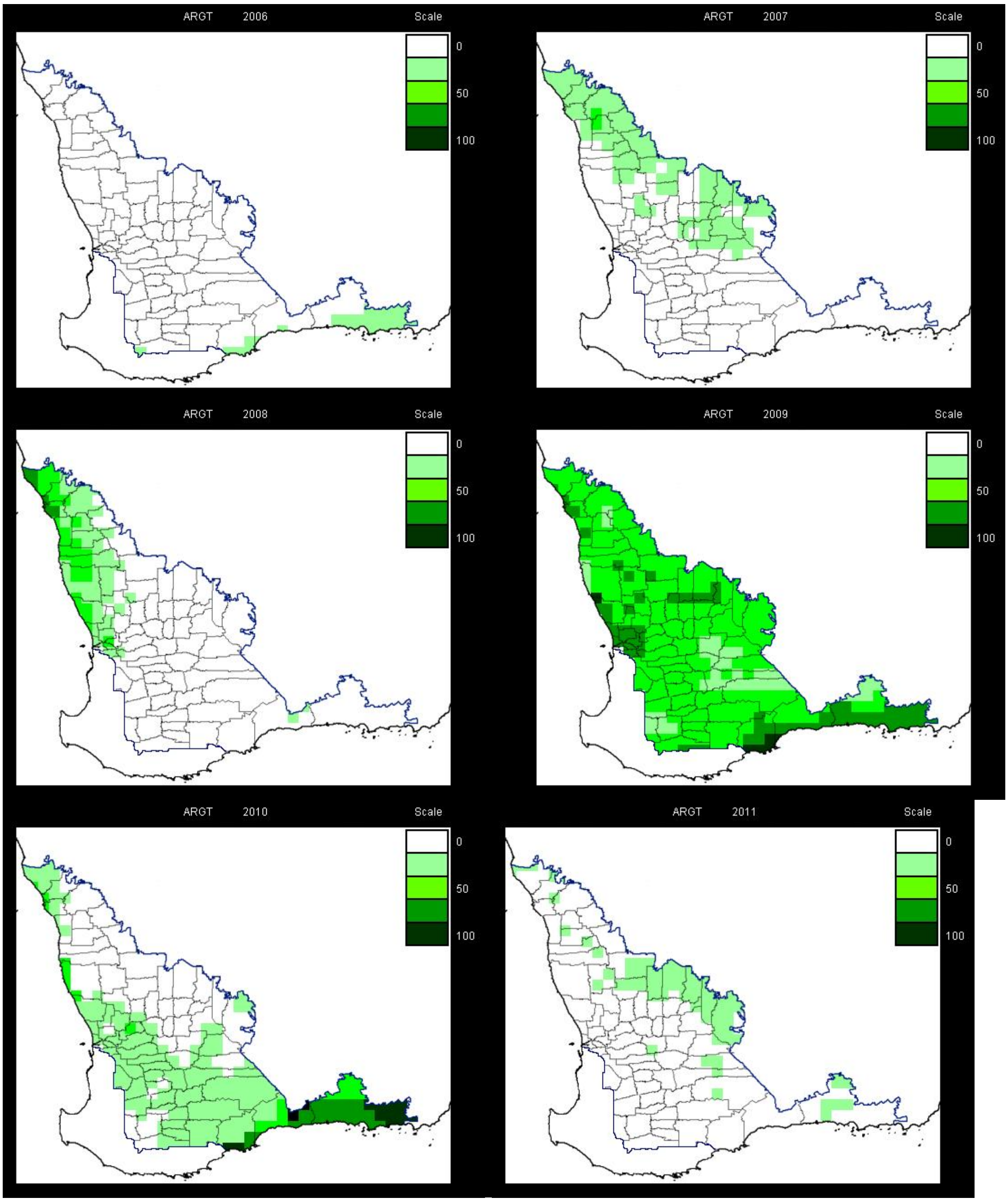

Figure 11. Predicted distribution of relative risk of incidence of *R. toxicus* in the agricultural region of south western Australia for the years 2006 to 2011.

From the multivariate analyses (PLSR), it was clear that prediction of risk of incidence of *R. toxicus* and in turn of ARGT must be made in two stages in a hierarchical system, called in this study S1 and S2. The hierarchical system in this case is first a prediction of risk in each year across the agricultural region of the south west of Australia, and second is a prediction of risk in that year by geographically (spatially) distinct areas in this region with a 4km resolution, distinct from Shire boundaries.

R. toxicus is the causative organism in ARGT. Risk of incidence of *R. toxicus* and potentially of ARGT is calculated as the number of cases of incidence of the bacterium *R. toxicus* divided by the number of observations in each site in each of six years of retrospective data, which comprise *ca.* 44% of analyses by DAFWA in those years. That is, incidence of the disease is compared with no incidence. Incidence of ARGT is estimated as EU (EIA units) > 20 from an immunoassay for *R. toxicus* by DAFWA, Masters *et al.,* 2006). 20EU is the maximum allowed by AQIS for hay to be exported (AQIS, 2005; Masters *et al*, 2007). 20EU equates to approximately 1 bacterial gall/kg hay. A bacterial gall is one which has been colonised by *R. toxicus*. Masters *et al* (2007) define risk of ARGT in oaten hay as shown in Table 3. Note that their definition of risk (galls/kg hay) refers to risk in terms of animal health of grazing livestock whereas RR(%) in the current study refers to risk of EU > 20 divided by the total number of observations of < 20 EU, where an EIA test of > 20EU means that the hay will be rejected for export.

Table 3. Risk of ARGT based on assay for *R. toxicus* using EIA as defined by Masters *et al*., 2007.

No risk	≤ 1 gall/kg hay	< 20EU
Very low risk	≤ 10 galls/kg hay	20 – 250 EU
Low risk	≤ 100 galls/kg hay	250 – 4000 EU
Moderate risk	≤ 300 galls/kg hay	4000 – 46000 EU
High risk	> 300 galls/kg hay	> 46000 EU

- **Prediction of relative risk (RR%) of incidence of *R. toxicus* is a two-stage or hierarchical process**

- First stage (S1): Risk of incidence of *R. toxicus* in a year

In this study risk is judged as incidence versus no incidence (based on number of observations), and is called relative risk (RR%). This is a common way to express incidence of disease in epidemiology. So, RR% is the number of samples with EU > 20 divided by the total number of observations in a PPD area. RR% was estimated in SaTScan for each geographic (spatial) location according to longitude and latitude, and then similarly for each PPD location using the concatenated data.

From PLRS analyses of the PPD data, the prediction of RR(%) based on weather observations in the PPD locations from January 1 until July 1 in each year is shown in Table 4. This is the algorithm used to predict RR% in a year across the entire south west agricultural region of Western Australia.

Table 4. Predictors and equation coefficients for the algorithm to predict RR(%) of incidence of *R. toxicus* in a year, based on weather information until July 1 in each year.

Predictors	**Equation**
Constant	52.69
Day of break of season ± 2 weeks	-0.11
Number of false breaks	0.24
Average maximum temperature in the first half of January	0.54
Average maximum temperature in the second half of January	-0.63
Average maximum temperature in the second half of March	-0.82
Average maximum temperature in the second half of June	0.78
Average rainfall (mm) in the second half of January (R_J2)	0.19
Average rainfall (mm) in the second half of April (R_Ap2)	-0.21
Average rainfall (mm) in the first half of June (R_Ju1)	-0.68

Using the algorithm in Table 4, RR (%) of incidence of *R. toxicus* (ARGT) for the years 2000 to 2005 for EU > 20 (with upper and lower confidence intervals) are listed in Table 5.

Table 5. RR(%) of incidence of *R. toxicus* in a year across the agricultural region of the south west of Western Australia.

Year	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**
RR (%)	47	46	35	41	36	32
Upper CI	49.2	47.5	36.5	42.5	37.5	33.5
Lower CI	44.8	44.5	33.5	39.5	34.5	30.5
Number of observations (PPD sites)	121	128	118	140	103	61

The average incidence of ARGT (RR%) of between 32 and 47% for all sites across the agricultural region of south-western Western Australia emphasises that *R. toxicus* is endemic in this region. It does *not* indicate that all properties or paddocks in each PPD area have recorded incidence of *R. toxicus*. The prediction of incidence of disease is significantly linear (RR(%) predicted = 0.88 + 0.98 RR (%) observed, n = 712, R^2 = 0.97). The RMSEP (standard error of prediction) from PLRS is 0.75.

RMSEP is an average error in a multivariate regression, and is different from the conventional error used in univariate linear regression (residual standard deviation). RMSEP is an estimate of the error in an unknown sample, predicted using the prediction algorithm. The approximate confidence interval of the prediction can be estimated as *ca.* twice the RMSEP, at the 95% confidence level. This is illustrated in Figure 12.

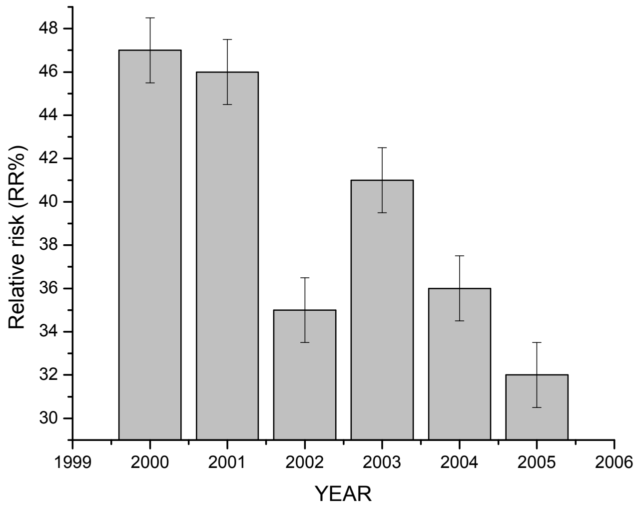

Figure 12. Relative risk (RR%) of incidence of *R. toxicus*, and by implication risk of ARGT. The error bars are estimated confidence intervals (2*RMSEP).

On the basis of these data it is proposed that RR(%) of incidence of *R. toxicus* for the agricultural region of south west Western Australia be categorised as shown in Table 6. It is proposed that on the DAFWA website this categorisation be shown.

Table 6. Categories of risk (RR%) of incidence of *R. toxicus* for the first stage (S1) of prediction.

Category of risk	**Range in predicted RR(%)**
Low	< 33.5
Moderately low	33.6 – 37.5
Moderate	37.6 – 45.5
Moderately high	45.6 - 47.5
High	> 47.6

This categorization makes:

- 2005 a low risk year
- 2002 and 2004 moderately low risk years
- 2003 a moderate risk year
- 2000 and 2001 moderately high risk years

It must be recalled that these categories are based on RR(%) of EU > 20, which is considered to be no or very low risk (Table 3, Masters *et al.*, 2007). That is, oaten hays will be rejected for export with EU > 20 but the margin for risk to health of livestock is wider than this.

Weather variables to discriminate between-year (the first stage, S1) and across PPD sites (the second stage, S2) RR(%) risk are designated or calculated as follows:

a) Months are designated as follows:
    - J - January
    - F - February
    - Mr – March
    - Ap – April
    - Ma – May

- Ju – June
- Jl – July
- Au – August

b) TM_*1 is average maximum temperature for the first 15 days of the month
c) TM_*2 is average maximum temperature for the remaining days of the month, regardless of leap years
d) TI_*1 is average minimum temperature for the first 15 days of the month
e) TI_*2 is average minimum temperature for the remaining days of the month, regardless of leap years
f) R_*1 is the average rainfall in the first 15 days of the month
g) R_*2 is the average rainfall in the remaining days of the month, regardless of leap years
h) Day of break of season is based on Chapman and Asseng (2001). Calculations of estimated false breaks in the season made use of their model where false breaks were estimated from rainfall and potential evapotranspiration in each month of the year from January to May. That model was extended to identify the day from January 1 of the season break. The calculation is a linear model. The number of false breaks is identified as # false breaks in any one year.
i) Break of season is expressed as plus or minus two weeks of the long term average (LTA, 1970 to 2000) (Brk ± 2). In this analysis LTA from Silo ‘data drill’ were assigned to the closest PPD. To use LTA ± 2 weeks was chosen from local experience. The years to use to calculate LTA were based on recent evidence of a significant decline in rainfall in southwestern Australia since *ca*. 1970 compared with previous years, as part of work on climate change in south-western Australia by the Indian Ocean Climate Initiative (IOCI, 2005). Collation of SILO ‘data drill’ data and calculations from them were made with algorithms written in MatLab. The calculations of break of season, of false breaks and of rainfall until each event were validated with the data used by Chapman and Asseng (2001) (Asseng *pers. com.*), and on-farm records for 2000 to 2005 from two growers, one near New Norcia and one near York. The simulations using the model of Chapman and Asseng agreed well with these recorded information.
j) EK - evapotranspiration using K_p and K_c factors developed by DAFWA (the ‘new’ model, see Methods)
k) G - greenness index developed by DAFWA (see Methods)
l) S - soil moisture developed by DAFWA (the ‘new’ model see Methods)

- **Second stage (S2): Prediction of risk by geographical location within years.**

This prediction is a second level prediction after prediction of the year risk (Tables 7 and 8). It is an hierarchical procedure. It assumes that in the future weather conditions will be similar to the six years of historical data used in this study. The prediction is for export cereal (principally oaten) hays where the RR(%) is likely lower than for livestock producers managing hays and pastures in the southwest of Australia (Masters *et al*. 2007). Despite this, it will not reduce the need for producers to take action to reduce their individual risk.

There are five levels of categorization of risk (RR (%), Table 6). Because there were no years in the study period that gave predicted RR greater than the fifth level of risk, the following predictions given for the remaining four are shown in Table8.

Table 7. Categories of risk (RR%) of incidence of from the first stage (S1) of prediction by year.

Risk category	Years used to predict risk by geographical location
Low	2005
Moderately low	2002 and 2004
Moderate	2003
Moderately high	2000 and 2001

That is, there are four prediction equations to predict within a year which geographical locations are at which level of risk (RR%). The algorithms used are shown in Table 8.

Table 8. Predictors and equation coefficients for the algorithms to predict RR(%) of incidence of *R. toxicus* in years, based on weather information until July 1 in each year.

Risk category	**Years used to predict**	**Predictors of RR (%)**	**Equation coefficients**	**R^2 of prediction**	**RMSEP (error of prediction)**
Moderately	2000 and			0.53	6.72
		B_0	265.44		
		TM_Mr1	-5.88		
		TI_F1	3.55		
		R_Ju2	-0.98		
		EK_Mr1	11.12		
		G_Ap2	-19008.60		
		S_AP2	2.88		
Moderate	2003	B_0	0.00	0.65**	5.72
		# of false	4.14		
		TI_Mr1	-11.64		
		TI_Ap1	16.39		
		R_Ju1	6.12		
		R_Jl1	-0.96		
Moderately	2002 and	B_0	-19.85	0.70***	6.82
		TI_Mr1	3.57		
		R_J1	-5.55		
		R_Ju1	-7.36		
		R_Ju2	-10.41		
		EK_Ap1	0.95		
		S_Mr1	-3.18		
		S_Ju1	0.04		
Low	2005	B_0	-24.81	0.59****	2.78
		Break day	0.20		
		R_Mr2	5.96		
		R_Ma1	-0.09		
		G_Ma2	-0.09		
		G_Ju1	0.50		
		S_Jl1	0.00		

*excluded PPD site 10018 as an outlier
**excludes PPD site 10624 as an outlier
***excludes three samples as outliers
****excludes PPD site 8067

An example of a prediction of RR(%) is shown in Figure 13. It is for years 2000 and 2001 combined, for which the risk category for those years is rated as moderately high. In these two years there is the poorest prediction of RR(%) (R^2 = 0.53), yet in these two years the prediction is statistically significant ($p < 0.05$).

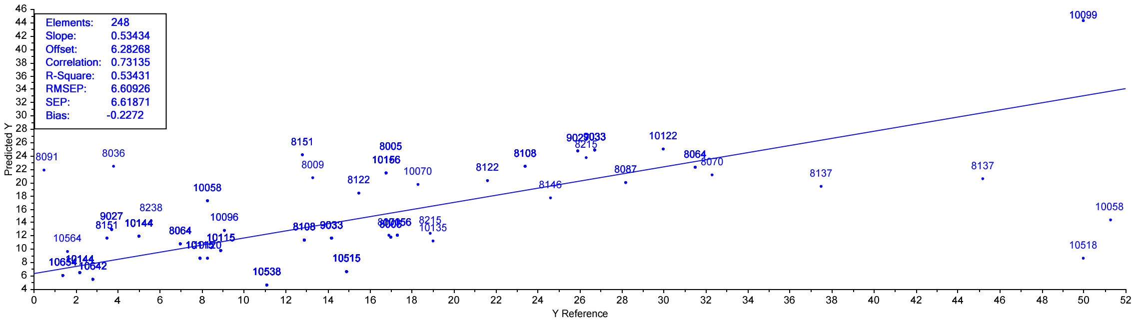

Figure 13. Example of prediction of RR(%), for the years 2000 and 2001. Numbers on the figure are PPD sites (n = 248 in this example).

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