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Risk analysis for biological weed control – simulating dispersal of *Sclerotinia sclerotiorum* (Lib.) de Bary ascospores from a pasture after biological control of *Cirsium arvense* (L.) Scop.

Meindert D. de Jong[1], Graeme W. Bourdôt[2,*], Geoff A. Hurrell[2], David J. Saville[2], Hans J. Erbrink[3] & Jan C. Zadoks[4]

[1]*Biological Farming Systems, Wageningen University and Research Centre, Marijkeweg 22, 6709 PG Wageningen, The Netherlands (E-mail: meindert.dejong@biob.dpw.wau.nl);* [2]*New Zealand Pastoral Agriculture Research Institute Ltd., P.O. Box 60, Lincoln, New Zealand (E-mail: graeme.bourdot@agresearch.co.nz);* [3]*KEMA Power Generation and Sustainable, P.O. Box 9035, 6800 ET Arnhem, The Netherlands (E-mail: j.j.erbrink@kema.nl);* [4]*Herengracht 96 C, 1015 BS Amsterdam, The Netherlands (e-mail: jczadoks@euronet.nl)*

*(*author for correspondence: Phone: 64 3 983 3973; Fax: 64 3 983 3946)*

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Abstract

Biological control of *Cirsium arvense* (L.) Scop. in pasture by the plurivorous plant pathogenic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary may result in the formation, escape and aerial dispersal of ascospores, creating an additional disease risk in down-wind market garden crops. To determine the width of a safety zone for a pasture subjected to this form of weed control, we simulated the spatial pattern in the ratio of added (due to biocontrol) to naturally occurring airborne ascospores (due to market garden crops) around a 1ha virtual biocontrol pasture under either sheep or dairy cattle gazing over a 91-day emission period in 1996 in Canterbury, New Zealand. This was achieved using a unique combination of two computer models; SPORESIM-1D (for spore escape from a vegetation source) and PC-STACKS (a modern Gaussian plume model for dispersal beyond a source). Plumes of dispersing ascospores were modelled for each hour of the emission period for both the virtual market garden and biocontrol sites, and the aerial density of the ascospores was averaged over the period. Assuming that a 1:1 ratio of added to naturally present spores is acceptable, no safety zone was necessary for either of the modeled pastures. A ten-fold ratio (1:10 added to natural) necessitated safety zones of 300 and 150 m for the sheep and dairy pasture respectively. Uncertainties associated with extrapolation of this conclusion to individual pasture management scenarios, and to other years and climatically different regions are discussed.

1. Introduction

The plant pathogenic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary has potential as a mycoherbicide for the control of *Cirsium arvense* (L.) Scop., *Ranunculus acris* (L.) and other weeds in pastures in New Zealand (Bourdôt and Harvey, 1996; Bourdôt et al., 1995; Cornwallis et al., 1999; Harvey and Bourdôt, 2001). However this pathogen has a wide host range including many economically important crops (Pennycook, 1989) and artificially increased inoculum in treated pastures may increase the disease risk in these crops downwind of the biocontrol site. Quantification of this risk is necessary for an informed judgement about the safety and management of this form of biological weed control.

Soilborne sclerotia and airborne ascospores are the most important forms of inoculum in *S. sclerotiorum* (Abawi and Grogan, 1979; Adams and Ayers, 1979; Schwartz and Steadman, 1978) giving, respectively,

temporal and spatial dimensions to crop disease risk; free soilborne mycelia are relatively unimportant (Cook et al., 1975; Huang and Kozub, 1993) and may be ignored. Considering the temporal dimension (Bourdôt et al., 2000), showed that sclerotium populations formed in sheep-pastures on a range of soils in the Canterbury region of New Zealand after biological control of *C. arvense*, decay to 'natural' levels on average within 4 years, after which time a susceptible crop planted at the site would incur a lower disease risk from the surviving sclerotia added by the mycoherbicide than from the naturally occurring sclerotia.

The sclerotia that are formed by the biocontrolled *C. arvense* plants over-winter in the soil beneath the pasture (Bourdôt et al., 2000). In the following spring (September, October and November) surviving sclerotia form apothecia at the soil surface that in turn release ascospores into the air within the pasture (Bourdôt et al., 2001). Depending mainly upon wind speed and pasture leaf area density, a fraction of these spores escape from the pasture canopy (de Jong et al., 2002) and disperse beyond the biocontrol site adding to the natural levels of airborne *S. sclerotiorum* spores in the neighbourhood of susceptible crops. Using an exponential decay model describing the gradient in the density of ascospores deposited downwind of a sheep-pasture treated with *S. sclerotiorum*, a safety zone of 8 m has been derived as the downwind distance at which the deposited densities of added and naturally occurring ascospores were equal (Bourdôt et al., 2001). The applicability of this safety zone under environmental conditions different from those prevailing during the experiment is questionable because spore liberation, transport and deposition may vary particularly with wind and air turbulence (McCartney and Fitt, 1985) as well as with vegetation density.

In this paper we take a simulation approach to determining the safety zone, accounting for the effects of fluctuating environmental conditions (temperature, solar radiation, wind speed, pasture leaf area density) on the escape and dispersal of *S. sclerotiorum* ascospores from a pasture that has been treated with the fungus for weed control. We illustrate how hourly estimates of 'ascospore escape fractions' generated by the computer model SPORESIM-1D (de Jong et al., 2002) can be used to estimate the 'source' term for a Gaussian plume model, PC-STACKS (Erbrink, 1995b) to provide (1) estimates of the 'natural' aerial spore densities at a market garden source as a result of natural infections in the market garden crops and (2) the 'added' spore densities in the air beyond a mycoherbicide source of *S. sclerotiorum* ascospores. In a contour plot of the ratio of added:natural ascospores around a virtual biocontrol site, we locate the contours for which the aerial density of ascospores dispersing from the biocontrol site, averaged over a three-month emission period, is equal to, or 10% of the median for the market garden source, giving safety zones for ratios of added to natural spores; 1:1 and 1:10.

2. Materials and methods

In this section two models are described that were used together to determine a safety zone around a pasture treated with *S. sclerotiorum* for control of *C. arvense*. Specific methodological details are also given for a particular analysis leading to the definition of a safety zone for a market garden area in Canterbury, New Zealand.

2.1 *SPORESIM-1D – a model for the escape of fungal spores from pasture*

The predecessor of SPORESIM-1D was developed to simulate aerial transport of the basidiospores of *Chondrostereum purpureum* within and beyond a forest canopy. The program has been adapted to simulate the dispersal of *S. sclerotiorum* ascospores in a pasture following biological control of *C. arvense* (de Jong et al., 2002). Like its predecessor, SPORESIM-1D is based on the gradient transfer theory (K theory) of turbulent diffusion (analogous with the molecular diffusion of gases) (Di-Giovanni and Beckett, 1990), and determines the density of spores in horizontal layers within the vegetation source, and the proportion escaping the vegetation canopy (Figure 1). Its mathematical description and validation have been reported previously (de Jong et al., 2002). SPORESIM-1D is written in Visual Fortran Version 6, and is available at http://www.dpw.wageningen-ur.nl/biob/organis/sporesim.htm.

The virtual pasture in the model measures 100 by 100 m and is divided into five horizontal air layers (layers 1 to 5 in the 30-layer SPORESIM-1D model shown in Figure 1). In layer 1, ascospores of *S. sclerotiorum* are released into the air after being ejected from apothecia that have developed on the sclerotia deposited on the soil after the death of the weed, e.g. *C. arvense*, following treatment with a *S. sclerotiorum*-based mycoherbicide. Some of the

ascospores in layer 1 are deposited on the ground or on pasture plants, and thus removed from the air. In all of layers 1 to 5, ascospores are transported vertically upwards, downwards by sedimentation, horizontally in the downwind direction, and are deposited on plant surfaces. In the other layers (6 to 30), all processes except deposition occur.

Parameter values for SPORESIM-1D are obtained from existing knowledge. The dispersal unit in the virtual grassland described by SPORESIM-1D, the airborne ascospore, is characterized by a sedimentation velocity, v_s, and a deposition velocity, v_d. The architecture of the vegetation layer is characterized by the vertical profile of its leaf area index, *LAI* (units of leaf area/unit of ground surface). Wind speed and turbulent diffusion for each layer are calculated from the wind speed u_r at a reference height above the crop (Goudriaan, 1989). SPORESIM-1D generates spore densities (# spores/m^3) in all of its air layers (1 to 30) at their respective heights above ground (0.0 m to 1.5 m). Horizontal (downwind) and vertical (upward) escape fractions are calculated together with deposition on soil and plants.

A simple model for the vertical escape fraction, E_v, was derived from the results of simulations with SPORESIM-1D (de Jong et al., 2002), in which E_v increases with increasing mean wind speed, u, and decreases with increasing pasture leaf area index, *LAI* (leaf area/ground area);

$$E_v = \exp\left[-b\frac{LAI}{\sqrt{u}}\right] \quad (1)$$

This simplification of the model was found to explain 99% of the variance in the escape fraction values generated for a wide range of wind speed and pasture *LAI* by simulation using SPORESIM-1D (de Jong et al., 2002), and is used for the analysis described in the current paper.

Horizontal escape is also modelled in SPORESIM-1D (Figure 1), but is negligible at the scale of a 100×100 m pasture and is therefore ignored in this paper.

2.2 *PC-STACKS – a model for the long-distance dispersal of atmospheric pollutants*

To describe the pathway of pollutants through the air, it is important to describe two main items. First, a description of the meteorological processes in the atmospheric boundary layer is necessary in order to predict the location of deposited pollutants, their concentrations in the air, and the rate of changes in these concentrations. Second, the form of the concentration gradients should be described mathematically in 3-dimensional space.

Dispersion theory started with G.I. Taylor (Taylor, 1921) who described the behaviour of particles in homogeneous turbulence. This analysis proved to be very worthwhile and was taken as the basis for many recommendations. Pragmatic formulations based on this concept and on fitted measurements (Pasquill, 1976) appeared to be more reliable than other approaches, especially for estimating the value of the lateral dispersion parameter σ_y (Irwin, 1983). However, more empirical formulations, which express σ_y and σ_z (the vertical dispersion parameter) as functions of distance for each of a number of 'stability categories' (Briggs, 1973; Pasquill, 1961) became more popular (although less accurate), partly because they do not require turbulence data as input.

The simplest approach for simulating the long-distance dispersal and mean densities of airborne particles, including pollen and fungal spores, over a defined time period (e.g. a particular spring) is the Gaussian plume model (Di-Giovanni and Kevan, 1991; McCartney and Fitt, 1985). In The Netherlands it was the basis of the national dispersion model, NATMO, one of the first computer programmes used for the simulation and management of air quality. It was written in Fortran for the mainframe system (DEC10) of Wageningen University in 1983 and was applied in spore dispersal studies in *Chrondrostereum purpureum* to help determine a safety zone between fruit-tree orchards and forests in which this fungus was to be used as a mycoherbicide for controlling the shrubby weed *Prunus serotina* (de Jong, 1988; de Jong et al., 1990a, b).

In the 1990s a more physical approach led to the evolution of the model STACKS, that did not use the stability classification and simple schemes of NATMO, but coupled dispersion directly to physically meaningful parameters. More fundamental scaling parameters for the atmospheric boundary layer were used in continuous functions to calculate turbulence, σ_y, σ_z and the height of the boundary layer with some parameters fitted on the basis of dispersion experiments (Erbrink, 1989, 1991a, b, 1994a, b, c, 1995a; Erbrink and Bange, 1991; Erbrink et al., 1994; KEMA, 1994; van Ham et al., 1998). This way of modelling was superior to other classification-based models and more suitable for calculating hourly mean concentrations. In 1997 STACKS was accepted as

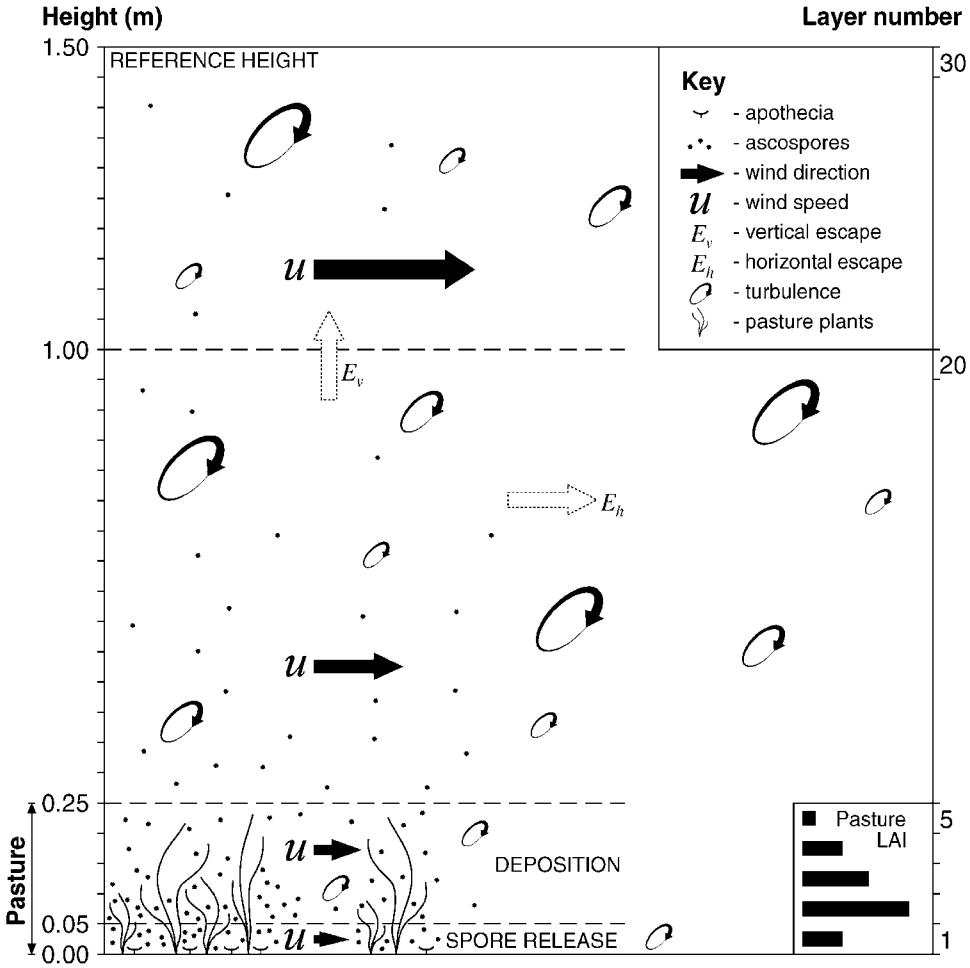

Figure 1. SPORESIM-1D, a multi-layer model for the transport of *Sclerotinia sclerotiorum* ascospores within, and escape from, a pasture sward. The air within and above the pasture, to a reference height of 1.5 m for wind speed, is divided into 30 layers. Ascospores are released by apothecia in the bottom layer. Deposition onto pasture plant surfaces can occur in layers 1 to 5. The fraction escaping vertically (moving vertically out of layer 20) is a function of turbulence, wind-speed (u), and the rate of deposition onto soil and plant surfaces (v_d); the latter is a function of pasture leaf area index (*LAI*) that varies vertically within the pasture. Figure reproduced with permission Journal of Applied Ecology (de Jong et al., 2002).

the New National Dispersion Model after completion of validation studies (Erbrink, 1995a). Comparing the results of STACKS with full scale field dispersion experiments with gases, the model was able to describe down-wind hourly average concentrations within a factor of 2 or 3; daily and yearly averages were more accurately predicted, within 20–50% and 10–25% respectively.

While STACKS was a research model, PC-STACKS version 4.1 became available for a wide range of users. PC-STACKS is an advanced Windows-based air quality management tool developed by the energy consulting company KEMA (Arnhem, the Netherlands). Here we have applied this advanced model to the dispersal of airborne ascospores of *S. sclerotiorum*.

In the Gaussian plume model implemented in PC-STACKS version 4.1, and illustrated in Figures 2 and 3, the atmospheric density of particles, C, at x metres downwind of a hypothetical emission source, is given by

$$C_{(x,y,z,H)} = \frac{PQ}{2\pi\bar{u}\sigma_y\sigma_z} \times \left[e^{\left[\frac{-(z-H)^2}{2\sigma_z^2}\right]} + e^{\left[\frac{-(z+H)^2}{2\sigma_z^2}\right]} \right] \times e^{\left[\frac{-y^2}{2\sigma_y^2}\right]} \times c_{ls} \quad (2)$$

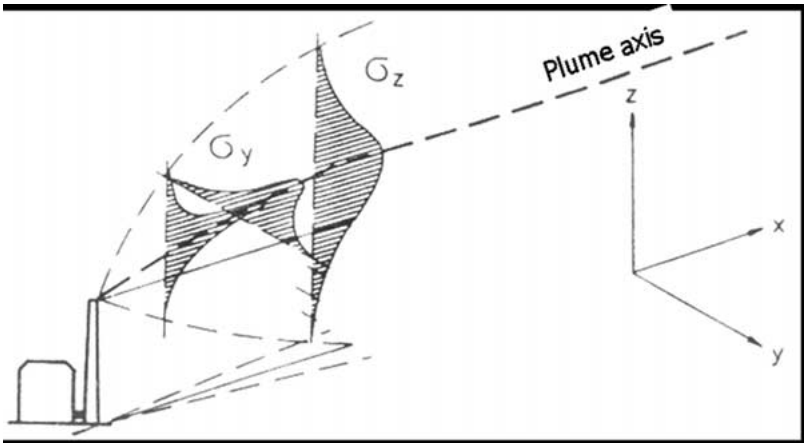

Figure 2. The Gaussian plume model with the horizontal or cross-wind (σ_y) and vertical (σ_z) dispersion terms illustrated. Figure reproduced with permission (Erbrink, 1995a).

where y is horizontal or cross-wind distance (m) from the plume axis (at right angles to this axis), z is height (m) above ground, H is the effective source height (stack height plus plume rise, m), P is the inversion layer penetration fraction, Q is the emission rate of the source (particles/s), $\bar{u}$ is mean wind speed at plume level (m/s), c_{ls} is a reflection term for multiple reflection against the top and bottom of the boundary layer, and σ_y and σ_z are respectively horizontal (cross-wind) and vertical dispersion parameters and are functions of turbulence parameters and wind speed together with the distance from source (x). Stability is directly related to turbulence and can vary from highly unstable, giving rise to large values for the dispersion terms, to stable when the dispersion terms are minimal (Figure 3).

Using a time series of hourly values of the input variables (emission, wind speed and direction, air temperature, solar radiation, cloud cover), as is common in air pollution studies (Dorrestein, 1981), PC-STACKS calculates, for the period of interest, the average hourly concentration of airborne particles at distances down wind from a source (Erbrink, 1995a). From these results, averages over other time scales can be obtained, such as daily or yearly mean concentrations. Subsequently, contours (lines of equal particle density (ascospores in our case)) can be drawn around the virtual source (s).

3.3 *Determining a safety zone for pasture treated with* Sclerotinia *sclerotiorum*

The question posed was "how near to land growing susceptible market garden crops (lettuce, celery, cabbage, broccoli, carrots, and others) can this form of biological weed control be practiced in either sheep or dairy pasture without 'unacceptably' increasing the disease risk to these crops?" In essence the approach taken in determining this 'safety zone' was to (1) simulate the concentration of 'naturally-occurring' *S. sclerotiorum* ascospores in the air above an area of market garden crops, (2) simulate the concentration of mycoherbicide-derived ascospores beyond a biocontrol source in both sheep and dairy pasture, and to (3) locate in 2-dimensional space around the two biocontrol sources, the concentration contour of added spores that equates to the median concentration in the market garden area (1:1 ratio of added to natural spores) and to one tenth of the median market

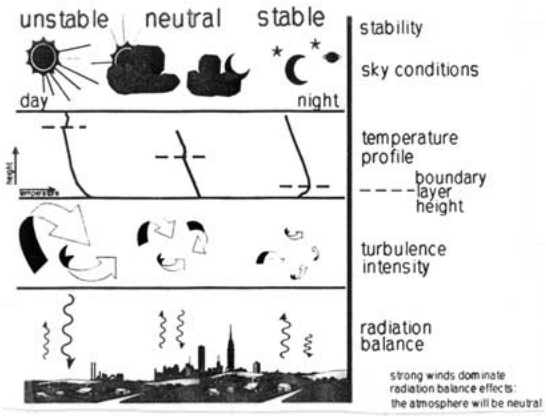

Figure 3. The atmospheric stability categories; neutral, stable, and unstable based on cloudiness and wind speed (Pasquill, 1961) as applied in PC-STACKS. Figure reproduced with permission (Erbrink, 1995a).

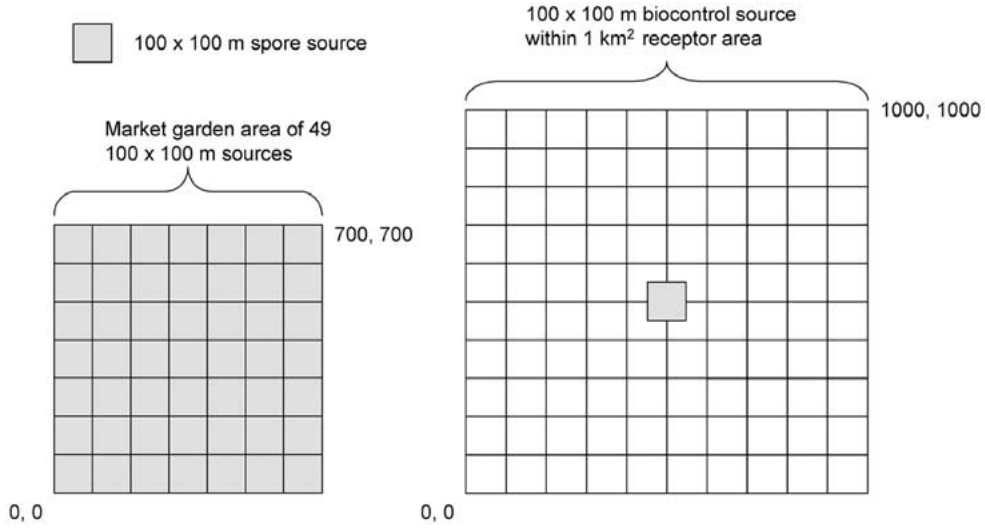

Figure 4. Spatial environments chosen for the simulations conducted using PC-STACKS to model the aerial concentration of *Sclerotinia sclerotiorum* ascospores in (1) a 700×700 m market garden area and (2) within a 1×1 km receptor area beyond a central 100×100 source in pasture where *S. sclerotiorum* has been applied to control *Cirsium arvense*.

garden concentration (1:10 ratio). These contours are the safety zones for their respective ratios of added to natural ascopores for sheep and dairy pasture.

The virtual market garden area was defined (in PC-STACKS) as a 7×7 square matrix of 49 sources each of 1 ha (100×100 m) in which 'naturally occurring' *S. sclerotiorum* ascospores are formed by natural infections in the market garden crops (Figure 4). The virtual pasture in which *S. sclerotiorum* has been applied as a mycoherbicide was defined as a single 1 ha (100×100 m) source of 'added' *S. sclerotiorum* ascospores (Figure 4).

In order to conduct the simulations leading to the definition of safety zones for the use of *S. sclerotiorum* for weed control in sheep and cattle pastures, two sets of input data values were passed to PC-STACKS.

Firstly, five meteorological variables for the 91-day period from 1 Sept 1996 until 30 Nov 1996, the period of the year when sporulation occurs in pastures (Bourdôt et al., 2001) (from weather station #H32641 at Latitude 43 39 S, Longitude 172 28 E and 11 m above sea level), were generated in an MS Excel spreadsheet and supplied to PC-STACKS in formatted text files. These variables were; hourly values of solar radiation (SR, watts/m^2), cloud cover $(S_{theoretical\ \max} - SR)/S_{theoretical\ \max}$, air temperature (°K), wind speed (u, m/s) and wind direction. These data enabled hourly estimates of P, $\bar{u}$, σ_y, σ_z and H in Equation (2) in PC-STACKS. Starting from these measured meteorological data, values for wind speed and turbulence were calculated by PC-STACKS at the appropriate transport and dispersion level. The roughness of the surface (set here as 'intermediate') is an important parameter determining the vertical profile of wind speed in the atmosphere and the degree of mechanical turbulence. These meteorological values largely determine the rate of dispersion. In addition, PC-STACKS was instructed that the land type over which dispersion of the spores was to take place was 'grazed grassland' located at 172° East and 34° South (Canterbury, New Zealand).

Secondly, the source term Q (spores/s), for Equation (2) in PC-STACKS, was calculated for each hour of the 91-day period in the MS Excel spreadsheet. The resulting time series of values were also supplied to PC-STACKS in formatted text files; one for each of the three simulation scenarios (market garden, sheep pasture, dairy pasture. Q was calculated as

$$Q = R_{spor} \times a \times E_v, \tag{3}$$

where R_{spor} is the release rate of ascospores from apothecia in the pasture or market garden sources (spores/m^{2}of ground surface/s), a is the area of the source (m^2), and E_v (from Equation (1)) is the proportion of the released spores escaping the pasture or market garden sources. R_{spor} was calculated as

$$R_{spor} = S \times A \times f, \tag{4}$$

where S is the density of sclerotia (#/m^2) in the soil at the source in the autumn, A is the size of the

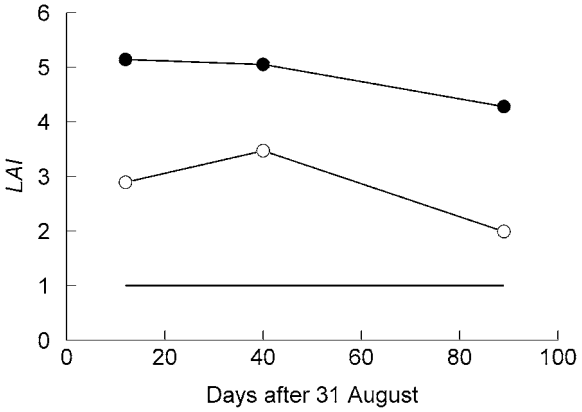

Figure 5. Progressions of *LAI* used in Equation (1) to simulate sheep pasture (○——○) and dairy pasture (●——●) sources of *Sclerotinia sclerotiorum* ascospores following biological control of *Cirsium arvense*, and market garden sources (——).

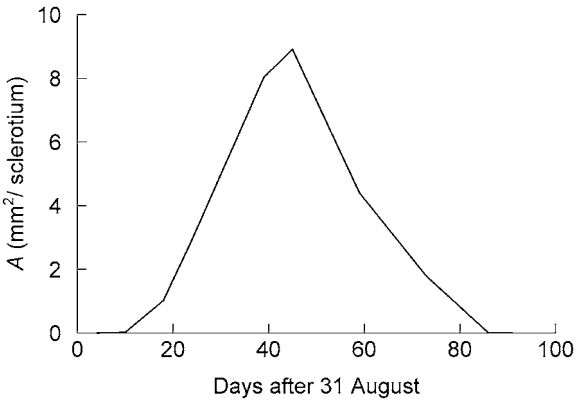

Figure 6. The progression of apothecial surface area *A* (mm^2/ sclerotium) used in Equation (4), constructed by linear interpolation between data values measured in a non-grazed sheep pasture in Canterbury (Figure 5d in Bourdôt et al. (2001)).

sporulating apothecial disc surface (mm^2/sclerotium) and f is the flux of ascospores from the apothecia (spores/mm^2 of disc surface/s).

The escape fraction was calculated hourly by Equation (1) for the 91-day period with parameter b = 0.934 (de Jong et al., 2002). Hourly mean wind speeds, u, for this period, at a height of 6 m above ground, were obtained from the Lincoln-Broadfields meteorological station (station #H32641).

LAI in Equation (1) was set to a constant 1.0 for the market garden area; a value that allowed for the fact that sporulation could occur on bare soil (*LAI* = 0), between widely spaced rows of vegetable crops (*LAI* = 0), and under crop canopies (*LAI* > 1) (Figure 5). For the sheep and dairy pasture sources, *LAI* was obtained by linear interpolation between the *LAI* values measured in sheep and dairy pasture in Canterbury during the 1996 spring and summer period (de Jong et al., 2002) (Figure 5).

The size of the sheep and dairy pasture sources, and of each of the 49 market garden sources making up the virtual market garden area, a in Equation (3) (specified as 'area' sources in PC-STACKS), was, as mentioned earlier, set at 100×100 m (1.0 ha). The density of sclerotia in the soil at these sources, S, in Equation (4), was set at 125 for sheep and dairy pasture and 8.8 m^2 for market garden sources based on previous measurements in sheep pasture and market garden soils in Canterbury (Bourdôt et al., 2000).

The progression of apothecial surface area, A in Equation (4), during the 91-day period (Figure 6), was derived by linear interpolation between data values measured in a non-grazed sheep pasture in Canterbury (Figure 5d in Bourdôt et al. (2001)). These data indicated that apothecia formation, and hence sporulation, is restricted in pasture in Canterbury to the 3-month period, September–November, and that apothecial surface area reaches a maximum in mid October. This pattern of apothecia formation was assumed to apply equally in the dairy pasture and market garden sources.

The flux of ascospores, f in Equation (4), from the apothecial surface given by A, followed a diurnal pattern; nil during the night and reaching maxima late morning on frostless and mid afternoon on frosty days according to data obtained previously in a sheep pasture (Figure 9 in (Bourdôt et al., 2001)). In the model f was considered to behave the same in dairy pasture, and market garden sources. A frosty day was defined as any day when the minimum temperature at ground level between 5 am and 9 am fell below 0 °C.

For each of the 49 'natural' market garden sources and the single 'added' biocontrol source, a plume of dispersing ascospores was generated for every hour from 1 Sept 1996 until 30 November 1996. Using all plumes (2,184 for both of the pasture sources and 107,016 for the market garden area), hourly average concentrations of ascospores beyond the pasture (within a 'receptor' area of 1000 m × 1000 m) (Figure 4) and within the market garden area (700 m × 700 m) (Figure 4) were calculated for a receptor height above ground of z = 1.0 m for the 91-day period. The median of the averages over the 7 × 7 matrix of sources representing the virtual market garden area was determined. Contour lines were then drawn within the 1000 m × 1000 m receptor area surrounding the virtual biocontrol pasture representing two ratios of

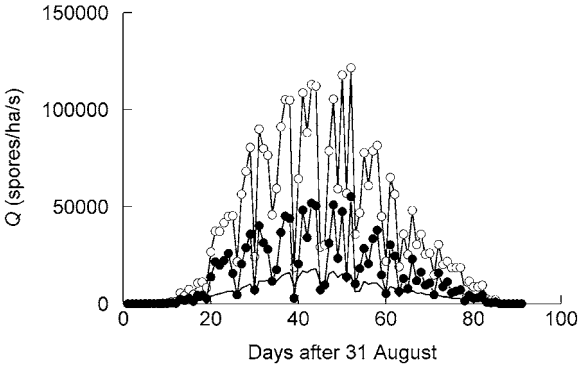

Figure 7. Daily means of hourly values of the source term, Q (spores/ha/s), calculated as in Equation (3), for the sheep pasture (○——○), dairy pasture (●——●) and market garden sources (——) of *Sclerotinia sclerotiorum* ascospores.

'added' (due to biocontrol) to 'natural' (due to market garden area) spores, 1:1 and 1:10.

3. Results

The daily means of ascopore emission, Q (ascospores/ha/s), varied throughout the simulation period (Figure 7). This variation was a result of the underlying temporal variation in apothecial surface area A (Figure 6), ascopore flux f, and the escape fraction E_v (Equations 3 and 4), the latter driven by the imposed *LAI* profiles (Figure 5) and hourly variation in wind speed, u. Of the two biocontrol pasture sources, Q was lower with dairy cattle grazing than with sheep due to a lower escape fraction in dairy pasture, which in turn was due to the higher pasture *LAI* in the dairy pasture (Figure 5). In the market garden source, Q was much lower than in either of the biocontrol pasture sources notwithstanding the assumed low *LAI* of 1.0 and therefore a relatively high escape fraction. The market garden Q was lowest because of the relatively low density of soilborne sclerotia in market garden soils (S = 8.8 cf. 125 in biocontrol pastures) giving rise to low values of R_{spor} in equation (4).

The simulated aerial density of *S. sclerotiorum* ascospores generated by naturally diseased crops within the virtual market garden area is illustrated as contours of equal density relative to the median density (Figure 8). The median of the 49 average densities generated by PC-STACKS, one value per 1 ha cell in the 7 × 7 cell matrix, was 2,880 × 10^6 ascospores/m^3 of air at a height of z = 1.0 m. Ascospore density varied asymmetrically across the

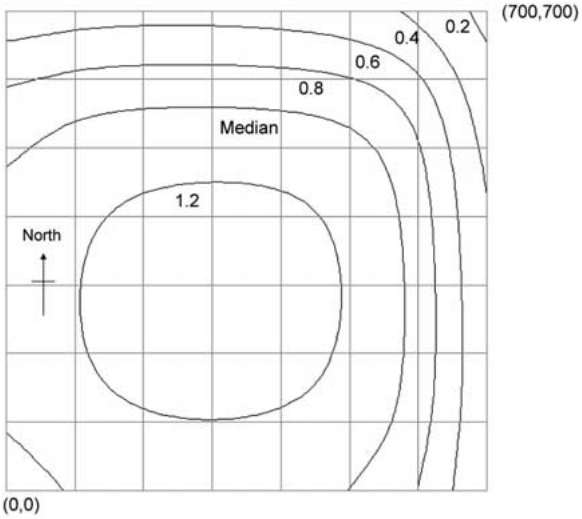

Figure 8. Simulated density of naturally occurring *Sclerotinia sclerotiorum* ascospores in the air at a height of z = 1.0 m in a 700 m × 700 m virtual market garden area modelled as 49 adjacent 1 ha sources of equal emission in Canterbury, New Zealand. The contours represent the median density and fractions of the median.

49 ha area as a result of the prevailing NE wind in Canterbury during the spring period.

The contour line for ascospores dispersing from the virtual sheep pasture biocontrol source at the density equal to the median density in the virtual market garden area (1:1 added to natural), was located within the boundaries of the source. The contour representing the 1:10 ratio of added to natural ascospores was located at greater distances from the biocontrol pasture source; 280 m SW and 100 m NE (Figure 9). This asymmetry is a result of the prevailing NE wind.

The contour line for ascospores dispersing from the virtual dairy pasture biocontrol source representing the density equal to the median density in the virtual market garden area (1:1 ratio of added to natural), was also located within the biocontrol source. The contour representing the 1:10 ratio was located beyond the biocontrol pasture source (but closer than for the sheep pasture) at 120 m distance in the SW direction and 60 m in NE direction, an asymmetry again due the prevailing NE wind (Figure 9).

4. Discussion

In this paper we have attempted to provide an objective basis for answering a particular question within the framework of a risk analysis for *S. sclerotiorum*

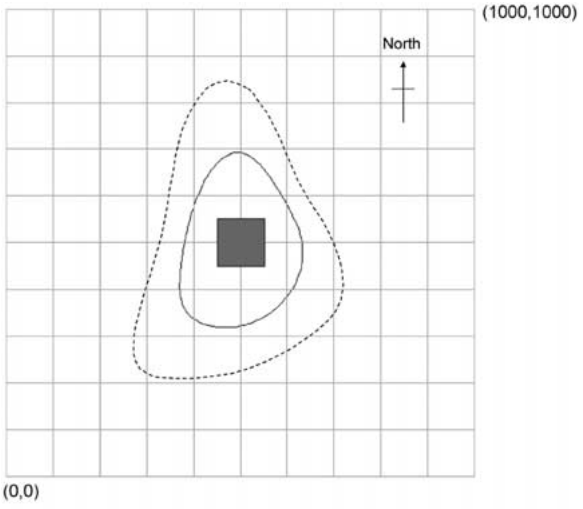

Figure 9. Safety zones for a 1:10 level of risk for sheep (- - - - - -) and dairy (——) pastures in Canterbury, New Zealand, treated with *Sclerotinia sclerotiorum* to control *Cirsium arvense*. The central square represents the 1 ha biocontrol source of ascospores. The contour lines represent the position where the ascospores dispersing from the biocontrol site, at a height above ground of z = 1.0 m, are at a density equal to 10% of the median for the market garden area.

(Lib.) de Bary when used in the biological control of the weed *C. arvense* (L.) Scop. in pastures in the Canterbury region of New Zealand. The question posed was "how near to land growing susceptible market garden crops can this form of biological pasture weed control be practised without 'unacceptably' increasing the disease risk to these crops?" The probability of disease in these crops could be increased above the natural level (due to naturally occurring inoculum) by ascospores coming from the pasture undergoing biocontrol. In Canterbury the air above market garden land contains ascospores of *S. sclerotiorum* throughout the year, and there are more spores on average above this land than above pasture (Bourdôt et al., 2001). This indicates that there is a relatively high natural risk of disease from this pathogen in market garden crops.

The approach taken in this paper is to consider the ratio of added to natural ascospores in the air above market garden crop land (de Jong et al., 1990a, 1999), and to make a judgement about the width of a safety zone on this basis. The distance from a pasture undergoing biocontrol of *C. arvense* by *S. sclerotiorum* at which the density of ascospores has declined to the density of ascospores occurring naturally in the air above market garden crops, i.e. ascospore density is doubled, may be acceptable as a safety zone (as in de Jong et al. (1990a, 1990b)). On this basis a safety zone would have been unnecessary for the sheep and dairy pastures modeled here (Figure 9). Furthermore, the distance beyond the sheep pasture biocontrol source where the density of the ascospores is 10% of the market garden level (1:10 ratio of added to natural ascospores) would define a risk averse safety zone; the results indicate that 300 m in any direction would have been adequate in 1996 (Figure 9). In the case of dairy pasture this 1:10 ratio distance was halved (Figure 9), a result of the greater spore-trapping ability of the dairy pasture due to its higher *LAI* (de Jong et al., 2002). This dramatic effect of pasture *LAI* on downwind spore density, suggests that withholding grazing during the sporulation period (September–November) in the year following an application of *S. sclerotiorum*, when ascospore emission is maximal (Bourdôt et al., 2001), would be an option for reducing any risk to a non-target crop. It also implies that the grazing strategy practiced in a particular sheep or dairy pasture after biological weed control with *S. sclerotiorum* may either promote or reduce the downwind concentration of *S. sclerotiorum* spores, thus either widening or shrinking the 1:1 and 1:10 average safety zones.

The safety zones calculated here are likely to be conservatively wide because in the model, the apothecial surface area per sclerotium, A, at the biocontrol pasture sources (and at the market garden sources) was based on data measured in a non-grazed sheep pasture (Figure 6). Under grazing it is probable that a proportion of the apothecia produced at the biocontrol pasture sources will be destroyed by treading. Although we have no supporting data for such an effect, any such damage would reduce the apothecial surface area per sclerotium. If for example apothecial surface area A (in Equation 4) is halved at the biocontrol sites in the presence of grazing animals, then Q and thus also ascospore density C (Equation 2), would be halved. This results in smaller safety zones than are indicated in Figure 9 since the aerial density of ascospores dispersing from the biocontrol sources would be 50% lower at all distances from the sources (Equation 2).

Since the safety zones estimated here are based on the average ascospore emission over the 91-day period in spring in Canterbury when *S. sclerotiorum* sporulates in pasture (Bourdôt et al., 2001) they can be expected to be reasonably robust. Nevertheless, they are also based on (1) the average sheep and dairy pasture and so don't account for extreme management scenarios that may either result in very low or very

high pasture *LAI*s, and (2) on just one year of meteorological data. Since *LAI* and meteorological conditions, are driving forces in both the escape (Equation 1) and dispersal (Equation 2) of the ascospores, safety zones could vary substantially between alternative pasture management strategies and between different years and climatic regions. In addition to these sources of variation, there may also be between-year variation in the length of emission period (Figure 6) and the seasonal pattern of the release rate of ascospores, R_{spor} (Equation 4), which would also contribute to between-year variation in the safety zone.

Because of these uncertainties, the safety zones calculated here will not be applicable to all pasture management scenarios, regions and years. Exploration of the effects of these sources of variation on the width of the safety zone is the subject of a further study. However, based on the current estimates, it does appear that a mycoherbicide based on *S. sclerotiorum* could be employed safely for weed control in sheep and dairy pastures in Canterbury when the meteorological conditions prevailing in the following growing season are similar to those of 1996. A precedent has been set in The Netherlands using a 'relative risk' approach where the Dutch Plant Protection Service permitted the use of *Chondrostereum purpureum* for controlling *Prunus serotina* provided that there is a distance of at least 500 m between the biocontrol site and commercial fruit growing (de Jong and Scheepens, 1985). Later a more risk-averse stance was taken by the Dutch PPS. It was decided that large *P. serotina* stumps must be covered with litter to reduce the level of fructification and hence spore escape and down wind density (Scheepens and Lotz, 1994), a use restriction analogous to withholding grazing during the brief sporulation period in the case of using *S. sclerotiorum* to control *C. arvense* in pasture.

In summary, in this paper we have linked two validated models, the SPORESIM-1D spore escape model (de Jong et al., 2002) and the PC-STACKS atmospheric dispersion model (Erbrink, 1995a), to determine a safety zone for the use of the fungus *S. sclerotiorum* as a mycoherbicide in pasture. In air pollution studies this combination of emission and immission modelling is common practice, but in the field of biological risks it is rather unique (de Jong, 1992). While PC-STACKS has been used earlier in risk assessments, e.g. accidental releases from nuclear power plants and from chemical industries, it has not previously been used in biological risk assessment. The results from PC-STACKS are accepted by authorities, because the model is based on modern physics and has achieved the status of National Model in the Netherlands. Since the dispersion of fine biological materials such as spores does not differ from the dispersal of fine non-biological particles, it is expected that the PC-STACKS model provides a good description of spore dispersion; this hypothesis is confirmed by a recent study (Spijkerboer et al., 2002). Nevertheless, the combination of the two models presented here for biological risk studies should be tested with a series of field experiments in which, for example, spore densities are measured downwind of a source of known strength and compared with model predictions. Furthermore, the accuracy of the models could be tested by an uncertainty analysis of their inputs and parameters.

As mentioned in the introduction, limited testing of a safety zone based on deposition of spores on a short time scale suggested a narrow safety zone of 8 metres downwind of the biocontrol source using the criterion of a doubling of the upwind deposition rate (1:1 ratio) in a pastoral farming system (Bourdôt et al., 2001). This serves as a limited validation of the '1:1' safety zone of zero metres predicted for market garden areas in the current analysis.

We surmise that models as discussed here, possibly complemented by models for spore deposition and disease generation, are applicable to many biocontrol problems in agriculture. Comparable models might also be used in warning systems for pollen allergenicity.

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