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Does insect netting affect the containment of airborne pollen from (GM-) plants in greenhouses?

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Abstract Greenhouses are a well-accepted containment strategy to grow and study genetically modified plants (GM) before release into the environment. Various containment levels are requested by national regulations to minimize GM pollen escape. We tested the amount of pollen escaping from a standard greenhouse, which can be used for EU containment classes 1 and 2. More specifically, we investigated the hypothesis whether pollen escape could be minimized by insect-proof netting in front of the roof windows, since the turbulent airflow around the mesh wiring could avoid pollen from escaping. We studied the pollen flow out of greenhouses with and without insect netting of two non-transgenic crops, Ryegrass (*Lolium multiflorum*) and Corn (*Zea Mays*). Pollen flow was assessed with Rotorod® pollen samplers positioned inside and outside the greenhouse' roof windows. A significant proportion of airborne pollen inside the greenhouse leaves through roof windows. Moreover, the lighter pollen of *Lolium* escaped more readily than the heavier pollen of Maize. In contrast to our expectations, we did not identify any reduction in pollen flow with insect netting in front of open windows, even under induced airflow conditions. We conclude that insect netting, often present by default in greenhouses, is not effective in preventing pollen escape from greenhouses of wind-pollinated plants for containment classes 1 or 2. Further research would be needed to investigate whether other alternative strategies, including biotic ones, are more effective.

1 Introduction

Many GM plants are grown in greenhouses under contained conditions following the Cartagena Protocol on Biosafety (UNEP 2004) and to national legislations on contained use. For activities involving GMOs conducted in greenhouses Directive 2009/41/EC applies in the EU (EC 2009). In order to prevent pollen escape, national legislations among others, Europe and North America, require physical containment through individual bagging of inflorescences of transgenic wind-pollinated plants (Sparrow 2010; Traynor-Dann and Irwin 2001). This is a time-consuming and

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expensive measure. Therefore, there is an increased attention for alternative methods that would minimize pollen escape from GM crops (i.e., dissemination; EC 2009, Table I B point 7). Such methods would apply for provisions of containment safety classes 1 and 2, containment classes in which air flow with the outdoors environment is still allowed (EC 2009; Table II).

A wide variety of biotic containment methods like pollen sterility is under development (Kwit et al. 2011). However, a low-cost alternative could be using the physical preventing capacity of fine insect netting, as e.g., suggested by the Dutch Commission on Genetic Modified Organisms (COGEM 2007; Van de Wiel 2007). Here, we report on this investigation.

The extent of the problem that pollen escape from crops initiate is still debated (Gressel 2010). Two undesirable effects are often mentioned (Craig et al. 2008). Firstly, pollen can contaminate crops of the same species grown outdoors. A contamination, even very small, might be undesirable in the case of commercially grown crops. Inadvertently mixing, that is, the inclusion of non-approved genes for release in the environment is not permitted for food (Davidson 2010). As well, for feed recently a 0.1% threshold has been implemented in the EU for mixing of unapproved GMO's, which are commercialized in third countries or pending approval in the EU (EC 2011). A further ecological undesirable effect could result from plants hybridizing with compatible relatives in the vicinity of greenhouses or cultivated areas. Incidental formation of hybrids between cultivated plants and their wild relatives is well-documented for areas where they co-occur (Ellstrand 2003, 2011). Transgenes built into the crop through genetic modification might, as a result, become introgressed into the genomes of nearby wild relatives, potentially leading to range the expansion of the species (Chapman and Burke 2006; Hooftman et al. 2008; Pilson and Prendeville 2004). However, the associated hazard is not uniform for all species and is often not yet defined (Craig et al. 2008).

Insect-proof screens are commonly installed in greenhouses in many countries. They act as barriers that prevent insects from entering the greenhouse, hereby avoiding plant damage by herbivory and/or pathogen transfer (Teitel 2007). Potentially, these screens could also minimize pollen escape from the greenhouse. Although the mesh size that is used is much larger than the size of pollen, reduced wind speeds and altered turbulence near the netting could

provide a barrier for the pollen (Teitel 2007), minimizing pollen escape and thus realizing up to containment class 2 (EC 2009). Empirical data on the actual pollen flow from greenhouses is as yet very sparse. Exceptions are two related studies measuring the escape of Maize pollen from an open pipe-frame greenhouse covered with 1-mm mesh on all sides (Watanabe et al. 2006a, b). Watanabe et al. (2006a) estimated the outcrossing rates in 6,000 trap plants (white Maize variety) directly surrounding a pollen source of 200 yellow Maize plants in a duplex 1-mm-mesh covered pipe frame, adjacent to 200 uncovered black Maize plants in the open. Most outcrossing events on the trap plants occurred within 5 m of the central pollen donor area, and the mesh reduced the number of outcrossed kernels from 594 for black to 139 for yellow pollen donors, indicating that the mesh indeed reduced gene flow. However, it is difficult to extrapolate the results from this Japanese case study to the situation in other countries, like North-Western Europe, where this type of mesh covered pipe frames is uncommon. In the country of study, the Netherlands, predominantly the "Venlo" type greenhouses is employed, the one we also use for our experiments.

Our research specifically asks how the presence of insect netting affects pollen escaping from greenhouses. We tested this, using two non-GM monocots as model organisms, Westerwolds Ryegrass and sweet corn (Maize). We experimented with and without insect netting in the roof windows following standard practice of opening the windows under warm conditions. We also artificially increased pollen concentrations inside the greenhouse, by generating extra upward airflow directed to the roof windows using large fans simulating a "worst case scenario" of high pollen concentration in the greenhouse. Pollen concentration was monitored inside and directly outside the windows of the greenhouse using Rotorod® samplers. Finally, we sampled the pollen concentration at different heights inside the greenhouse.

2 Materials and methods

2.1 Greenhouse

The experiments were performed in and outside a 38-m^2-double-span greenhouse compartment. The

compartment is located in the northeastern corner of the greenhouse complex of the University of Amsterdam at Science Park Amsterdam (N52° 21.29′; E4° 57.52′). The greenhouse is of the Venlo-type with a gutter height of 4.50 m. The eight windows have an angle of 30° from horizontal and are situated at 4.80–5.30 m height (Fig. 1). Total greenhouse volume is around 175 m^3. Individual window surface area is 2.5 m^2, for eight windows per compartment this sums up to 20 m^2. The windows are normally covered by insect-proof netting with a mesh size of 400 × 450 μm (0.4 × 0.45 mm), providing a 30% reduction in ventilation relative to open windows, according to the manufacturer's specifications.
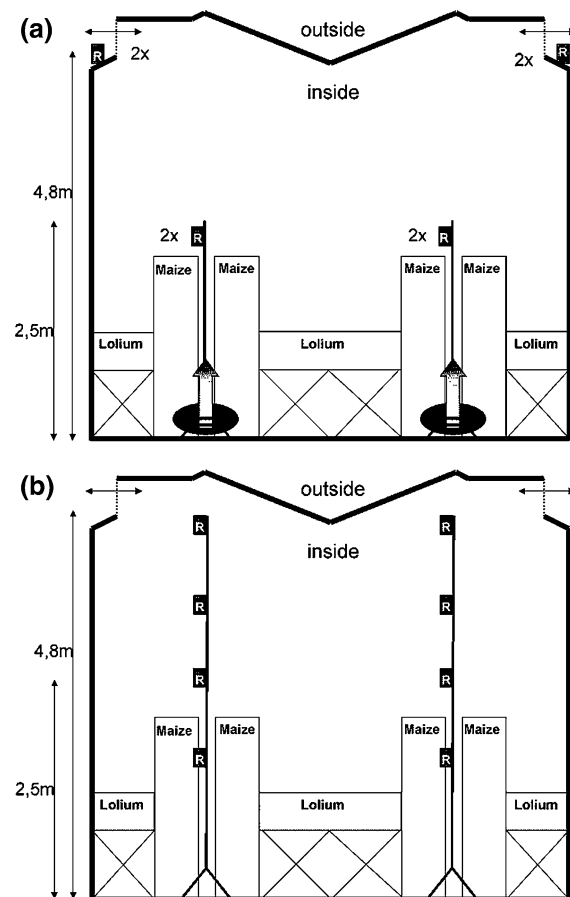
Fig. 1 Side view of the greenhouse compartment. Setup **a** was used to measure pollen escape; setup **b** to measure pollen dynamics inside the greenhouse under ambient conditions. Rotorod samplers are indicated by "*R*." The *large shaded arrows* indicate the direction of the induced airflow

2.2 Plant material

Model species were the wind-pollinated species Westerwolds Ryegrass (*Lolium multiflorum* Lam., ssp. *alternativum*) and sweet corn (*Zea mays* L., var. *rugosa*), further referred to as "*Lolium*" and "Maize." The *Lolium* was collected as sod cuts from cultivated fields of Barenbrug Holland BV in Leens, Groningen (N53° 21′; E6° 24′). Maize seeds were obtained from Gebr. Eveleens, Aalsmeer (2008-catalogue, item nr 522). Two subsequent series of plants were used resulting in two distinct flowering periods. The cultivars we used are non-GM for the manifold of practical and biosafety reasons.

The two species differ in pollen size and, therefore, also in weight. Maize has relatively large pollen grains (mean Ø 89 μm; Beug 2004), whereas *Lolium* has relatively small pollen (mean Ø 32 μm; Beug 2004). Pollen size is an order of magnitude smaller than the mesh. The *Lolium* sods were grown on 90 cm high tables, the surface totaling 12 m^2 (Fig. 1), and they flowered continuously during a nine-week period. The resulting pollen release height was 1.20–1.50 m. On the floor surrounding the tables, we placed 120 Maize plants (Fig. 1) resulting in a pollen release height of 2.50–3.00 m. Maize flowering peaked around mid-July.

2.3 Pollen traps

To measure the pollen concentration, Rotorod® samplers Model 20 (Multidata LLC) were used. Rotorods have a rotating arm that traps particles onto two rapidly spinning polystyrene rods. The samplers have a wide range of applications, from healthcare-related studies (Hugg et al. 2007; Hugg and Rantio-Lehtimäki 2007) to studies on the dispersal dynamics of plant species (Aylor 2005; Aylor et al. 2006; Van Hout et al. 2008; Spijkerboer et al. 2002).

Four Rotorods were placed inside the greenhouse at four different positions, just above the Maize inflorescences at approx. 2.5 m height, as well as outside the greenhouse, with four Rotorods attached to the roof just in front of the roof windows (Fig. 1a). The collector rods were treated with a thin layer of silicone grease, catching all small airborne particles they encounter. For practical reasons, the rods were running parallel to the airflow both inside (upward) and outside (facing the window) the greenhouse. Each collector

rod has an effective area of 1.52×22 mm and runs at 2,400 rpm. The total volume sampled per hour per collector rod is 1.3 m^3 (Multidata 2002).

2.4 Sampling scheme

Samples were taken during the flowering periods of Maize and *Lolium* from the July 23 to September 17, 2008. Sampling was restricted to non-rainy days, since windows are closed during the rain and the electronic equipment could not be used outside. Samples were taken between 9 am and 5 pm with a maximum of five runs per day. A single sample involved simultaneously running all eight Rotorods inside and outside the greenhouse for 60 min. Daily samples included three types of treatments:

- Non-disturbed ("ambient") conditions with natural air movements within the greenhouse and air vented through the roof windows.
- A "worst-case scenario" with artificial "induced" airflow: four vertically placed domestic fans (Proline Ø 40 cm, 1,800 Watt) blowing upward toward the windows were used to increase vertical airflow and turbulence.
- Outside background sampling: daily background samples were taken (with exception of the first week for logistic reasons) to estimate the background pollen concentration of all *Poaceae*. The latter measure was used, since pollen grains from *Lolium* are not visually distinguishable from pollen grains from most other *Poaceae* species. Greenhouse windows were closed during these measurements to prevent escape.

Insect netting was removed and placed back following a 5–6 day cycle to obtain several series with the absence or presence of insect netting.

2.5 Sample treatment and counting

Collector rods were analyzed with light microscopy (Leica; magnification 400×) after being placed in a stage adapter and stained with Calberla's stain (Benton Franklin Health District 2009; Multidata 2002). All *Lolium* and Maize pollen grains were counted on the entire collector rod area of 1.52×22 mm. Concentrations are reported as pollen capture per m^3 per hour.

2.6 Pollen grain distribution in the greenhouse

To assess the change in concentration with increasing vertical distance, two poles were placed on opposite sides of the greenhouse compartment, each equipped with four Rotorods at 2, 3, 4 and 5 m above the floor (Fig. 1b). Samples were taken with the windows either open or closed. This was repeated six times between August 27 and September 4, using eight Rotorods samplers under ambient conditions.

2.7 Statistical analysis

The data were analyzed using a generalized linear model. The statistical model fitted the pollen count outside (PCO) in each of the four outside Rotorods, depending on the average pollen counts inside (PCI) at 2.5 m height and the presence of insect nets, while correcting for the background pollen count (BPC) outside measured on the same day. The generalized model used was:

$$PCO_i \leftarrow \alpha PCI + \beta_i[\text{Rotorod}_i \times PCI] + \gamma[\text{Insect Net} \times PCI] + \delta BPC + \text{error}$$

For *Lolium*, the number of pollen captured was high hence a standard ANOVA method could be employed, that is, using the identity link, and minimizing the least-squares error for model fit. We employed a Type III Sums of Squares. Because statistics were performed with untransformed count data, α can be directly interpreted as the average ratio (slope) of the outside-to-inside pollen count, the β_i parameters allow for differences in this ratio among the four outside Rotorods at different positions, the γ parameter estimates the difference in the ratio due to the insect mesh, and δ corrects for potential effects of background pollen from other sources, determining the intercept of the model. Our approach has the advantage that there is no need to calculate any averages prior or after the statistical analysis.

The Maize pollen counts were much lower. Therefore, we employed a Type III ANOVA with Poisson error distribution and logarithm link function and minimized the derived errors in difference between observed and predicted counts given the Poisson distribution. The latter model is appropriate for counts containing many zero values. For the statistical tests, the procedures UNIANOVA and GENLIN of SPSS v17.0 were used, respectively (SPSS Inc., Chicago, IL).

3 Results

3.1 Background pollen capture

The average background pollen count (BPC) for Poaceae pollen was 2.2 grains per m^3/h, measured outside the greenhouse with closed windows. No background Maize pollen was found.

3.2 Lolium pollen escape

In contrast to the initial expectations, relatively *more* pollen were found outside the greenhouse in the presence of insect netting under ambient conditions. Although we identified a substantial variation in the counts in and outside the greenhouse, there is a clear relationship between in and outside pollen count (Fig. 2a), which is also confirmed by the statistical analysis (Table 1). The total statistical model explained 71.2% of the variation in outside counts. Our factor of interest, the presence of insect netting, was highly statistically significant under ambient conditions ($P < 0.001$; Table 1). Furthermore, the covariate of inside pollen count (PCI) was significant ($P < 0.001$) as was the effect of the covariate background Poaceae pollen, which is in accordance with our expectation (estimated parameter δ close to one, 0.957; $P < 0.05$). We found a consistent difference between the four outside Rotorods positions ($P < 0.001$).

The estimated ratio of outside-to-inside of airborne pollen concentration was 3.0% $\pm$ 0.4 (mean $\pm$ SE) without insect netting and 13.8% $\pm$ 1.5 with insect netting in place. It is not clear what caused this difference; we suggest it could be caused by changed airflow conditions. Note that the low ratio without netting is mostly attributable to two runs with an inside pollen count of over 1,000, coupled to—relatively—low outside counts. Possibly high flowering incidence coinciding with relatively cool weather conditions may have played a role here. In any case, these results do not contain statistical support for a *reduction* in the escape due to the insect nets under ambient conditions, as the estimated effect is in the opposite direction (Fig. 3a).

With fans activated both the inside and outside counts were higher than under ambient conditions (Fig. 2b). Under increased airflow, the total statistical model explained 62.2% of the variation. Effects of average inside pollen count (PCI) were again highly

(a) **Ambient conditions (*Lolium*)**
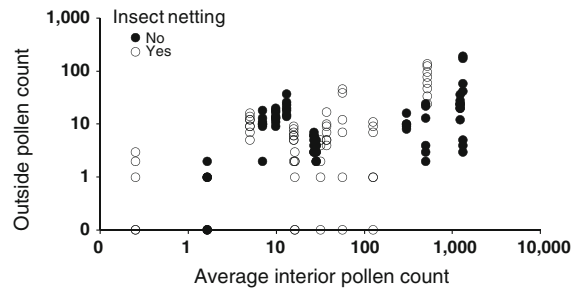
(b) **Induced airflow (*Lolium*)**
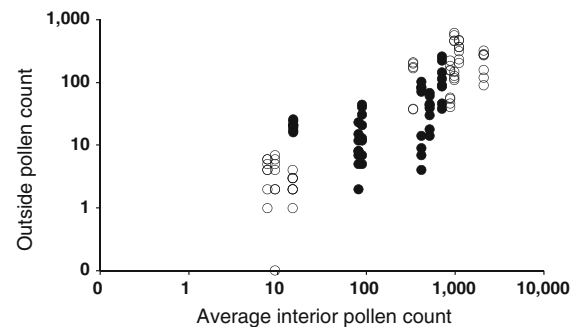
Fig. 2 Captured *Lolium* pollen outside the greenhouse *with* and *without* insect netting in front of the windows under **a** ambient and **b** induced airflow conditions. Each *data point* represents a pollen count of one of the four Rotorods outside the greenhouse, given the average pollen count within the greenhouse at 2.5 m height. Note the *data* are plotted on a logarithmic scale for visual reasons

significant ($P < 0.001$), whereas the effect of the covariate background Poaceae pollen was not significant (estimated parameter δ again close to one, 1.259). The estimated ratio of outside-to-inside pollen concentration was 13.0% $\pm$ 4.3 without nets and 16.9% $\pm$ 1.4 with nets. However, the difference due to netting presence, our factor of interest, was not statistically significant under the influence of an induced airflow, which can also be seen from the graph of the predicted values (Fig. 3b). We found a consistent difference in pollen counts for the four outside Rotorods ($P < 0.001$), indicating that position effects (possibly due to wind directions) were present.

3.3 Maize pollen escape

Maize pollen did generally not escape from the greenhouse under ambient conditions. The amount of airborne pollen at 2.5 m height was extremely low, and Maize pollen was only sporadically found outside

Table 1 Analysis of *Lolium* pollen escape using a type III general linear model ANOVA

Source of variation	Ambient conditions			Induced airflow conditions		
	df	MS	F	df	MS	F
Pollen concentration inside the greenhouse (PCI)	1	57,082	191.53***	1	482,799	42.26***
Background pollen concentration (BPC)	1	2,019	6.77*	1	1,962	0.17
Rotorod position × PCI	3	9,268	31.10***	3	26,956	2.36***
Insect mesh presence × PCI	1	24,605	82.56***	1	8,791	0.77
Residual error	144	298		104	11,425	

The interaction between insect mesh presence (yes/no) × inside pollen concentration (PCI) estimated the effects of insect mesh on the outside pollen count, given the average pollen count inside the greenhouse. Two conditions are separately tested: ambient and induced airflow conditions (activated fans)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

(a) Ambient conditions (*Lolium*)

(b) Induced airflow (*Lolium*)
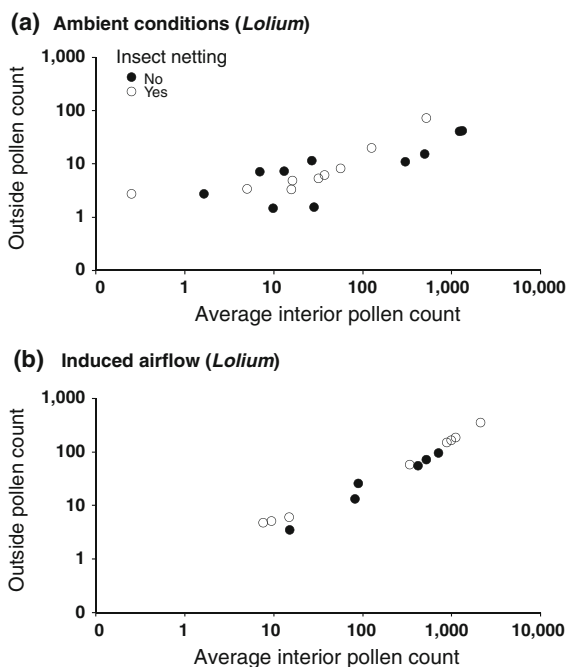
Fig. 3 Model predictions of estimated marginal means for *Lolium* pollen escape (averaged over the four outside Rotorods), *with* and *without* insect netting in front of the windows under **a** ambient and **b** induced airflow conditions. Note the *data* are plotted on a logarithmic scale for visual reasons

(Fig. 4a). The reason for the low numbers is probably caused by its relatively high pollen mass, although a low pollen production during parts of the sampling period may have played a role. No statistical significant effects were found under ambient conditions (Table 2). However, the power of the analysis was low, as in total only 10 pollen grains were found

outside and 55 airborne inside at 2.5 m ($n = 72$ rods), resulting in an average outside-to-inside ratio of $21.5\% \pm 8.0$ without netting and $14.1\% \pm 6.3$ with netting.

Under induced airflow conditions, insect netting did not reduce the pollen escape from the greenhouse, although this treatment was effective in increasing the number of airborne Maize pollen (Fig. 4b). The outside pollen concentration increased in chorus with the inside pollen concentration at 2.5 m, with estimates of $6.3\% \pm 2.5$ and $4.1\% \pm 1.0$ for the ratio outside-to-inside without and with netting, respectively. Furthermore, we found a significant effect of Rotorod position ($P < 0.001$, Table 2). In general, no statistically significant effect for the presence or absence of the insect netting was found under induced airflow for Maize (Table 2; Fig. 4).

3.4 Pollen flow dynamics within the greenhouse

Turbulence in the greenhouse caused by airflow through the windows seems important for the airborne pollen concentration and their layering within the greenhouse. Comparing airborne pollen concentrations during closed window conditions (i.e., no external influence) and open windows conditions revealed that both Maize and *Lolium* pollen concentrations were much higher when the windows were open (Fig. 5). Furthermore, the heavier Maize pollen grains remained closer to the floor, showing a pronounced decrease in pollen concentration from 2 to 3 m elevation. The lighter *Lolium* pollen grains show a much more gradual (but still considerable)

(a) **Ambient conditions (*Maize*)**

(b) **Induced airflow (*Maize*)**
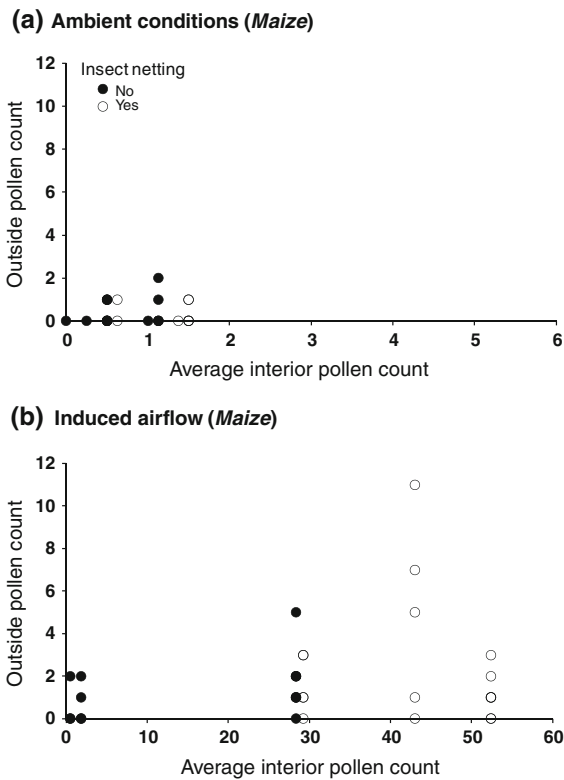
Fig. 4 Captured Maize pollen outside the greenhouse *with* and *without* insect netting in front of the windows under **a** ambient and **b** induced airflow conditions. Each *data point* represents a pollen count of one of the four Rotorods outside the greenhouse, given the average pollen count within the greenhouse at 2.5 m height. Note the order of magnitude differences in *x*-axis between both figures

change in concentration with increasing elevation. The steep decrease in pollen concentration in Maize is in agreement with the low escape rates we observed.
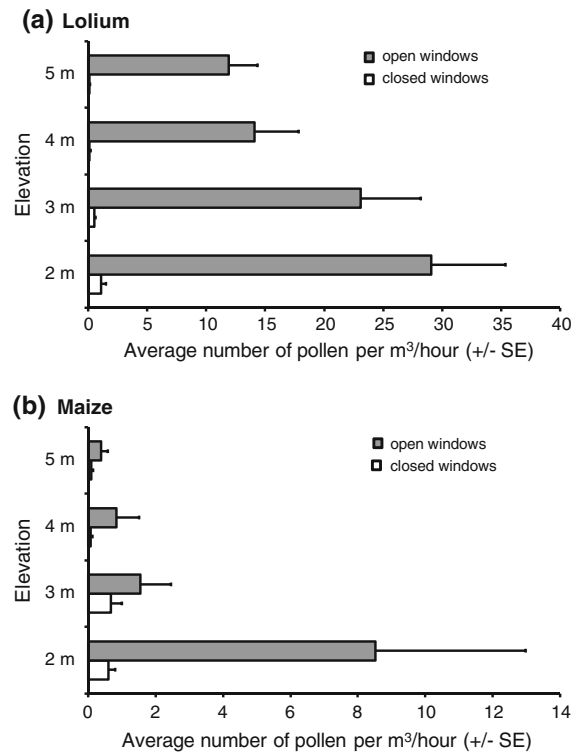
(a) **Lolium**

(b) **Maize**

Fig. 5 Pollen concentrations inside the greenhouse for **a** *Lolium* and **b** Maize at four different elevations, with the roof windows opened (with insect nets in place) or closed

4 Discussion

Minimizing pollen escape from GM plants grown under biosafety containment classes 1 and 2 (EC 2009) is essential since these greenhouses are still connected to the outdoors environment for climate regulation (Critten and Bailey 2002; Traynor-Dann and Irwin 2001). Such containment is similar to the classification

Table 2 Analysis of Maize pollen escape, using a generalized linear model with log link and Poisson errors

Source of variation	Ambient conditions		Induced airflow conditions	
	df	Wald chi-square	df	Wald chi-square
Pollen concentration inside greenhouse (PCI)	1	2.18	1	9.16**
Rotorod position × PCI	3	1.19	3	17.8***
Insect mesh presence × PCI	1	0.65	1	2.62
Residual (deviance)	66	38.0	42	82.1

The interaction between insect mesh presence (yes/no) × inside pollen concentration (PCI) estimated the effects of insect mesh on the outside pollen count, given the average pollen count inside the greenhouse. Two conditions are separately tested: ambient and induced airflow conditions (activated fans)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

according to Dutch regulation system: "PK 1" and "PK 2" (BGGO 2008). Transgenes in such greenhouses will often be still in the later stages of development and hence still lacking approval for release in the environment. Here, the question is investigated whether in-place insect netting could be effective as pollen barrier as was suggested (COGEM 2007).

In general, a considerable fraction of pollen that is released into the air in a greenhouse, and remains airborne, will find its way out through open roof windows, irrespective of the presence or absence of standard insect netting. We found no indication that pollen escape was in any way reduced by the turbulent airflow surrounding the insect netting, neither under ambient conditions nor under conditions with an artificially increased upward airflow. The observed pollen concentration inside the greenhouse at 2.5 m height as well as outside varied considerably from day to day for both *Lolium multiflorum* and *Zea mays*. Also, the range of values found for the ratio of *Lolium* pollen outside relative-to-the inside varied considerably. Differences in outside air temperature could not explain this variation (supplementary materials). However, increased wind speeds enhanced the pollen concentrations of *Lolium* both inside and outside the greenhouse, but in a similar way. No such correlations were found for Maize (supplementary materials).

4.1 Airflow dynamics within the greenhouse

Screening the windows with insect netting affects airflows inside a greenhouse in complex ways (Majdoubi et al. 2007; Shilo et al. 2004; Teitel 2007). Greenhouse screening by insect netting can cause a significant decrease in turbulence (Katsoulas et al. 2006; Kittas et al. 2008) depending on the type of netting. Bartzanas et al. (2004), Dayan et al. (2004) and Kittas and Bartzanas (2007) simulated airflow and temperature patterns under different regimes finding similar results. Shilo et al. (2004) suggested that air flows in the lower part of the greenhouse were mostly not directed toward the windows but rather toward the ground and to the sides. This indicates that a substantial fraction of pollen would end up adhered to plants, soil, pots and the floor, especially the heavy pollen. Indeed, the artificial upward airflow conditions under screened conditions increased the concentration

of pollen in our experiment, possibly also including older pollen of the previous days.

Optimal airflow within greenhouses aims to create homogenous temperature and growth conditions for plants (reviewed in e.g., Critten and Bailey 2002; Teitel 2007). Employing smaller mesh sizes would presumably be more efficient in blocking pollen, such as the filers experimented with for transgenic *Brassica* (Waschmann et al. 2010). However, this would also reduce the venting of the greenhouse and would soon make forced and filtered air management needed causing much higher costs.

4.2 Differences in the fate of pollen

We found large differences in the vertical distribution of Maize and *Lolium* pollen, as predicted from the difference in pollen size between the species (Beug 2004). The concentration of Maize pollen within the greenhouse decreases steeply with height, so that the escape rate is reduced. In fact, the bigger Maize pollen grains rarely reached the level of the roof windows, unless turbulence was (artificially) high. The lighter *Lolium* pollen travel much higher and more readily escape through the roof windows. For a quantitative risk assessment, it is, therefore, important to take pollen grain size into account. Of course, size also affects the fate of pollen after escape: heavier pollen travel less far (see e.g., Kuparinnen et al. 2007; Watanabe et al. 2006c). In general, the bulk of the escaping pollen will not reach a compatible plant at all, these being too far away, or the pollen may have died before pollination occurs.

The absence of Maize pollen in the background measurements indicates that there were hardly any flowering Maize plants in the neighborhood at the time of the experiments. Moreover, the phenology of commercially grown crops may not overlap with the phenology of the plants in the greenhouse. Species with light pollen and a locally common recipient population like *Lolium* would have a much higher likelihood of outcrossing than species such as Maize, given that Maize pollen are heavy, hardly escape from greenhouses and are carried less far in the air. For Maize, data on outcrossing rates related to distance between the crop fields are available (DeVos et al. 2005; Hooftman and den Nijs 2007), as well as on pollen movement through space (Aylor 2005; Aylor et al. 2006). A difference between pollen escaping

from greenhouses and from field crops is that the release height of pollen is higher, which might cause pollen to travel further. Whether this causes a significant alteration of the pollination–distance relationship is unknown.

4.3 Methodological constrains

For practical reasons, the treatments (netting/no netting) were performed during several periods of several days. Inevitably, this led to a difference in weather conditions during observations with and without netting and also to variation in flowering intensity of plants. However, in both treatments, there was a considerable range of observed values, and the design allowed us to directly compare inside and outside counts that were obtained simultaneously (i.e., paired observations). The identified consistent relationship between in and outside measurements confirms that this variation in pollen presence in time was not problematic.

Especially, Maize showed a clear peak flowering period in which it releases all of its pollen within a few days. Therefore, it was only possible to sample Maize pollen up to 10 days after the initiation of the flowering period, limiting the power of the analysis. An alternative approach would have been to use Maize plants of different ages, to spread the release of pollen more evenly in time. On the other hand, this might reduce the pollen density to lower levels, as in our setup there were distinct periods with a high pollen production. Wind also affected the Rotorod sampler collection efficiency. Although the Rotorod samplers perform very well compared to other pollen samplers (Heffer et al. 2005; Latorre et al. 2008) and certainly under influence of varying wind speeds, the maximum change in collection efficiency is still reported at 39% (Frenz 2000). Such noise increases the sample error and decreases statistical power. Furthermore, we captured pollen inside the greenhouse above the canopy to estimate the amount of airborne pollen and did not measure the total pollen production or release. Therefore, it is not possible from the present data to estimate the proportion of pollen produced that escapes through the roof windows. It is plausible that this proportion will be much lower than the relative ratio of pollen concentrations outside-to-those airborne inside at 2.5 m height, as most pollen will not reach this height.

4.4 Conclusion

We found no evidence for a reduction in proportion of pollen escaping from greenhouses with standard insect netting in front of the top windows. Studying pollen escape from greenhouses is rarely done as yet, since it is highly elaborate and prone to variation. Hence, comparing among studies is not yet possible. Likely, netting with a smaller mesh could be more efficient in blocking pollen. However, the more intensive regulating of the greenhouse environment could easily undo the efficiency gain of not bagging or caging plants individually.

In conclusion, insect netting, as mostly standard present in greenhouses, does not seem to minimize pollen escape from greenhouses. Hence, it is an unlikely option to obtain any containment level in line with current EU-legislation (EC 2009) when growing wind-pollinated (GM-) organisms. We suggest that more future potential might be in enhanced male sterility and other biotic containment strategies like transgene mitigation (Gressel 2010; Hooftman et al. 2011; Kwit et al. 2011).

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