Ventilatory impairment from pre-harvest retted flax

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ABSTRACT A new method of retting flax recently developed to suit the United Kingdom climate has allowed the reintroduction of flax growing to this country. The weed killer glyphosate is sprayed on the crop which then rets before harvesting six weeks later. The acute bronchoconstrictor responses of 11 normal subjects to dust from dew retted and from pre-harvest retted flaxes were compared in a double blind crossover fashion. There were no significant differences in the dust levels nor in the size of the dust particles in the experimental dust room. The decreases in pulmonary function after six hours of dust inhalation were significantly larger after pre-harvest retted flax dust than after dew retted flax dust (Δ FEV₁, -0.21 and -0.40 l; Δ MEF₅₀, -0.72 and -1.21 l/s; Δ sGaw (specific airway conductance), -0.17 and -0.65 kPa/s for dew retted and pre-harvest retted respectively). The subjects also reported more symptoms after inhaling pre-harvest retted flax dust. It is concluded that the acute bronchoconstrictor response to flax dust is increased by pre-harvest retting, suggesting an increased risk of byssinosis.

To process flax for its fibre, the plant stem needs to undergo controlled putrefaction of pectic substances that bind the tissues together. This process, which frees the fibres from the woody core, is known as retting. Most flax is dew retted by micro-organisms that grow on the plant while it is allowed to lie in the field after harvesting. The climate in countries such as the United Kingdom is unsuited to this method. Pre-harvest retting has recently been developed by the Lambeg Industrial Research Association, Lisburn, and the Department of Agriculture, Queen's University of Belfast, to suit the wetter climate.^{1,2} The weed killer glyphosate is sprayed on the mature plant six weeks before harvesting. The glyphosate is translocated throughout the plant, which desiccates and rets. Pre-harvest retting allows flax growing to be reintroduced to countries such as the United Kingdom where climatic conditions are unsuited to dew retting. A limited amount of flax for pre-harvest retting is already being grown in Northern Ireland, Scotland, and France on a commercial basis, but most of the flax processed is imported dew retted flax. It was thought that alterations in bacterial growth and the improved ventilation by retting while the plant was still standing in the field might reduce the development of bronchoactive toxins and possibly reduce the risk of byssinosis in flax workers, but in the

present experiments it was found that the acute bronchoconstrictor response of dust from pre-harvest retted flax was greater than that from dew retted flax.

Methods

SUBJECTS

The 11 normal subjects (six male, five female, aged 19-23) were screened to exclude allergy, asthma, and rhinitis. All gave informed consent and the Queen's University Ethical Committee gave their approval.

DUST

Six weeks before harvesting, 225 l/hectare of a 6.3 g/l solution of glyphosate ("Round up," supplied by Monsanto Limited) was sprayed at a pressure of 2.0 to 2.25 bar on the flax crop.³ The dust generated in the normal doubling process in a flax mill was collected into new clean dust bags. The doubler machine was carefully cleaned before use. Dust was collected separately when pre-harvest retted and dew retted flax were being processed. The amount of dust collected was 17 g/kg of dew retted flax and 12 g/kg of pre-harvest retted flax after hackling. This difference may be within the error of collection. The dust was sieved at 180 μ m and dispersed into a room in which a group of five or six subjects were seated.⁴ The dust levels and particle size distributions were recorded by Rotheroe & Mitchell and Batelle cascade analysers; the levels were typical of those in a dusty flax mill.

PROTOCOL

A double blind crossover comparison of the two dusts was made by dividing the 11 subjects into two groups with three men and three women in one group and three men and two women in the second group. Specific airway conductance (sGaw) was measured as the mean of ten measurements on each subject using a PK Morgan computerised whole body plethysmograph. After these measurements, five forced expiratory manoeuvres were recorded by a computerised McDermott spirometer and flow/volume curves constructed. In one subject flow rates were not recorded because of a technical failure. Measurements were made before and immediately after six hours of dust inhalation. Each group was allowed one week to recover from the previous dust.

STATISTICS

Each pulmonary function variable was taken as the mean of all the measurements taken (five forced expiratory readings and ten airway conductance readings). Mean decreases between before and after the two dusts were compared with paired *t* tests. The means after the two dust inhalations, ignoring the pre-exposure controls, were also compared with paired *t* tests. An analysis of variance was also carried out to test for interaction between the order of dust inhalations and their effect.

Results

DUST

Table 1 shows the mean dust levels for each dust.

These levels were monitored half hourly for the six hours on each of the two days for each dust. Each measurement was made in duplicate on two Rotheroe & Mitchell analysers. The standard errors shown are calculated from the 24 half hourly measurements. The table also shows the percentage by weight of the dust in each size range, as measured by the Batelle aerodynamic cascade impactor. Each percentage shown is the mean of one measurement on each of the two days for each dust. There was no significant difference in the total dust level or the particle size distribution between the two dusts. The load of dust on the turntable for dispersal was adjusted to keep the dust level in the room constant. The average load for the dew retted flax dust was 0.9 g/min and for the pre-harvest retted flax dust 0.8 g/min.

BRONCHIAL RESPONSES

Table 2 shows the mean control levels of lung function before the challenge with each dust; they are in good agreement. Every subject showed a fall in FEV₁ after six hours of inhaling dew retted flax dust and a larger fall in FEV₁ after six hours of inhaling pre-harvest retted flax dust. The FVC, MEF₅₀, and sGaw measurements gave qualitatively similar results for the subjects with one or two exceptions; the mean decreases appear in table 2. There were approximately twofold greater decreases after pre-harvest retted flax dust than after dew retted flax dust. The differences in these decreases were statistically significant except for MEF₅₀, which did not quite reach the 5% level, although the means after dust, ignoring the control readings, were significantly lower

Table 1 Mean (SE) dust levels and percentage particle size distribution by weight of dust in each size band

	Mean dust level (mg/m ³)	μ m						
		0–	0.5–	1–	2–	4–	8–	≥ 16
Dew retted	5.16 (0.16)	4.4	4.3	8.1	27.9	31.7	13.2	10.4
Pre-harvest retted	5.14 (0.16)	6.9	3.3	10.9	25.0	27.6	16.6	9.7

Table 2 Mean and mean decreases (SE) of five forced expirations and ten specific airway conductance measurements on 11 subjects before and after dust inhalation

	Dew retted	Pre-harvest retted	p
Before			
FEV ₁ (l)	4.087	4.069	
FVC (l)	4.607	4.612	
MEF ₅₀ (l/s)	5.937	5.822	
sGaw (l/kPa/s)	2.382	2.570	
After six hours minus before			
Δ FEV ₁ (l)	–0.210 (0.053)	–0.397 (0.063)	< 0.001
Δ FVC (l)	–0.102 (0.041)	–0.255 (0.051)	< 0.01
Δ MEF ₅₀ (l/s)	–0.717 (0.210)	–1.205 (0.264)	≈ 0.05*
Δ sGaw (l/kPa/s)	–0.167 (0.125)	–0.648 (0.140)	< 0.01

*MEF₅₀ after pre-harvest retted was significantly lower than after dew retted (p < 0.001).

after pre-harvest retted flax dust. This was partly explained by the failure of the control readings of MEF_{50} fully to return to normal one week after challenge with the previous dust. This is shown in table 3. The pre-challenge means on the second day showed decreases in FEV_1 , FVC, and MEF_{50} . These differences were small and not significant for FEV_1 and FVC, but the difference for MEF_{50} was appreciable and was statistically significant ($p < 0.05$). There was no apparent difference in the decreases in response to bronchial challenge between the two groups of different subjects who inhaled the two dusts in reverse order, and analysis of variance showed no significant interaction between the order of dust inhalation and response to dust ($p > 0.5$).

SYMPTOMS

The number of subjects reporting symptoms on a questionnaire that they completed after each dust challenge is given in table 4. There were more symptoms after pre-harvest retted flax dust inhalation. It was noticeable that two subjects from the group who inhaled the pre-harvest retted flax dust first and three from the group who inhaled it second spontaneously commented that the pre-harvest retted flax dust was more irritating.

Discussion

COMPARISON OF DEW RETTED AND PRE-HARVEST RETTED FLAX DUSTS

The decreases in lung function representing bronchoconstriction over six hours of dew retted flax dust inhalation were similar in these experiments to those which we have reported previously.⁴ Pre-harvest retted flax dust inhalation caused considerably greater bronchoconstriction as shown by all the measurements of lung function and by the recorded symptoms. The differences in lung function which were statistically most significant were the FEV_1 and sGaw measurements. The MEF_{50} differences were large but not statistically significant because of their greater

Table 4 Number of subjects with symptoms

	Dew retted	Pre-harvest retted
Nasal irritation	2	2
Throat irritation or cough	7	9
Chest tightness	4	6
Breathlessness	0	2
Nausea	1	2
Sweating or shivering or both	1	2
	15	23

variability, showing fourfold greater standard errors. Bouhuys *et al* have also found greater decreases in MEF_{50} than in FEV_1 and that the MEF_{50} measurement was more variable.⁵ The sources of variability of maximal expiratory flow rates have been described by Clement and van de Woestijne.⁶ In the present comparative trial between the two dusts the variation in control readings was increased by the incomplete recovery of the MEF_{50} before the second dust challenge.

Symptoms experienced with the pre-harvest retted flax dust were more frequent and generally more severe. The distribution of symptoms with both types of dust was similar to that found in managers from flax mills⁴ with the exception of two subjects who reported sweating, shivering, or nausea. If these symptoms are neglected there were more symptoms of airway effects (throat irritation, cough, and chest tightness and breathlessness) after the pre-harvest retted flax dust.

These differences were not due to the order of challenge with the two dusts because both groups, who inhaled the dusts in reverse order, showed the twofold greater decrease in lung function after the pre-harvest retted flax. The two flaxes differed in that the dew retted flax was grown in France and its variety is unknown. The pre-harvest retted flax grown in Northern Ireland was the variety Hera which is commonly grown in France. It is not thought that these differences are likely to be the cause of the differences

Table 3 Mean control values and decreases after dust inhalation in the two groups who inhaled the dusts in reverse order. Group A inhaled dew retted first; group B inhaled pre-harvest retted first.

		FEV_1	FVC	MEF_{50}	sGaw
Group A (n = 6)					
Dew retted	Before	4.28	4.90	6.03	2.30
	Δ	-0.23	-0.10	-0.95	-0.13
Pre-harvest retted	Before	4.20	4.86	5.51	2.66
	Δ	-0.43	-0.18	-1.16	-0.73
Group B (n = 5)					
Pre-harvest retted	Before	3.92	4.32	6.14	2.47
	Δ	-0.36	-0.28	-1.25	-0.55
Dew retted	Before	3.86	4.26	5.84	2.48
	Δ	-0.18	-0.10	-0.50	-0.21

found because we have challenged subjects with a range of common varieties of imported French flax and have never recorded as large a decrease in lung function as after the present pre-harvest retted flax.

The degree of retting differed slightly between the two flaxes. The pre-harvest retted flax was sandy brown in colour suggesting that it was under-retted compared with the greyer dew retted flax. This difference is unlikely to account for the greater bronchoconstriction because green under-retted flax has been found to be less byssinotic.⁷

The pre-processing of the two flaxes and other aspects of the experiments were identical for both flaxes. We therefore suggest that the differences were due to the new method of retting. This was unexpected because it was thought that the use of a chemical, combined with the better ventilated conditions for the plant while still standing in the field, would diminish the production and retention of toxins. Chemical retting using caustic alkaline solutions has been associated with a reduction in the acute bronchoconstrictor response to dust⁷ and in byssinosis.⁸ The mechanism of pre-harvest retting is not fully understood but it is not a simple chemical effect since the rate of retting after glyphosate is diminished in the presence of fungicides and abolished by autoclaving (PC Mercer, unpublished data). Brown and Sharma have demonstrated fungal enzymes that are capable of retting.⁹ The organisms and enzymes were the same as in dew retting, although the counts of the different species may vary (PC Mercer and TW Fraser, unpublished data). Culture and identification of organisms in pre-harvest retted flax dust is not yet available.

TOXICOLOGY OF GLYPHOSATE

By the end of the six week period required to allow the organisms to ret the flax, there is little glyphosate residue. Nevertheless the effects of inhalation of this chemical in man are unknown and it cannot be excluded as a cause of the increased pulmonary function impairment observed.

The manufacturers, Monsanto Ltd, have carried out extensive toxicological studies. In rats the acute oral LD₅₀ has been reported to be 5 g/kg.¹⁰⁻¹² A major postmortem feature is hyperaemia of the lungs.¹⁰ The LD₅₀ is lower by intraperitoneal injection (0.2 g/kg).¹⁰ The inhalation LC₅₀ for rats is 3.18 g/m³ for four hours inhalation (Monsanto, personal communication). Subchronic toxicity in rats tested by three months inhalation for six hours a day, five days a week, at concentrations of glyphosate of 40–200 mg/m³, showed no effects on survival, growth, biochemistry, or haematology. Mean lung and liver weights were decreased in the tested group compared with controls (Monsanto, personal communication).

The recommendation for oral human consumption is less than 6 mg/day for a life time.^{11 13} The residual level of glyphosate was estimated in flax fibre by Monsanto and reported as 6 mg/kg of flax fibre. The levels in the dust room should therefore have been less than 10⁻⁴ mg/m³ at a dust level of 5 mg/m³. About 2–3 m³ of air would be inhaled in the six hour period in the dust room. These levels are substantially lower than the toxicity studies on rats and the recommended human consumption rate. No inhalation studies in man have been carried out, however, and bronchoconstrictor effects in lower doses may have been missed in animals.

PATTERN OF RESPIRATORY IMPAIRMENT AFTER FLAX DUST

The decreases in FEV₁ and the larger decreases in MEF₅₀ after inhaling flax dust have been reported previously.⁴ The present study also showed decreases in sGaw. The decreases in conductance were smaller than the decreases in FEV₁ and MEF₅₀. This was not due to the bronchodilator effect of a previous deep inhalation¹⁴ because of the order in which the tests were carried out. Bouhuys and van de Woestijne also found this pattern of reaction by some subjects to hemp dust and suggested that it might be due to a greater effect on the small rather than the large airways.¹⁵ This interpretation is supported by the analysis of Mead *et al* who suggested that it is the resistance of peripheral airways and lung elastic recoil which determine maximal mid-expiratory flow rates.¹⁶ The decreases during constriction, however, may be more dependent on changes in collapsibility of larger airways.¹⁷

The subjects showed consistently large or small decreases after dew retted and after pre-harvest retted flax dust. The correlation coefficient between Δ FEV₁ after dew retted and after pre-harvest retted flax dust was 0.89 ($p < 0.001$). There is thus a spectrum of high to low responders. Bouhuys and van de Woestijne found that subjects who had a small or absent flow rate response to hemp dust did have a conductance response.¹⁵ They suggested that these subjects may have had a predominantly large airway response and that there were no true non-responders. The high proportion of fine dust after sieving and dispersal may possibly have modified our responses but we did not find greater conductance responses when the flow rate responses were smaller. Linear regression analysis of Δ FEV₁ and Δ sGaw showed a positive slope of 1.02 after dew retted and 1.14 after pre-harvest retted flax dust. The present experiments therefore do not support the finding of larger decreases in sGaw in those subjects with smaller decreases in FEV₁.

Recovery from both dew retted and pre-harvest retted flax dust inhalation was not complete after one

Ventilatory impairment from pre-harvest retted flax

week. This was statistically significant only for MEF₅₀. Symptomatically, three subjects reported symptoms lasting three to five days. These observations suggest that the effect of one day's exposure to dust may last for up to one week. It may be that the slowest peripheral parts of the lung to eliminate retained dust particles¹⁸ may also have the greatest effect on the MEF₅₀ measurement.

PREDICTION OF BYSSINOSIS RISK

The incidence in byssinosis in workers using this new method of pre-harvest retting could be determined only by a long term prospective trial. In the absence of such data it is predicted that repeated bronchoconstriction would occur with continuous exposure and that this would be harmful. By analogy, another noxious particulate inhalant, cigarette smoke, causes an acute bronchoconstriction¹⁹ and long term chronic airflow obstruction. Mill fever is a condition that occurs in a small percentage of subjects on first exposure to dust but tends not to recur on subsequent exposures, although Pernis *et al* and Cavagna *et al* have suggested a common mechanism for mill fever and byssinosis.^{20,21} Symptoms suggestive of mill fever occurred in two subjects after dew retted flax. They complained of shivering or nausea, or both, but body temperature was not recorded. The symptoms lasted longer and were more severe after pre-harvest retted than after dew retted flax dust, irrespective of which dust was inhaled first. That a transient first exposure effect on lung function is unlikely is suggested by the finding that the bronchoconstriction was slightly larger after the second challenge irrespective of which dust was inhaled (mean Δ FEV₁, after first challenge = 0.29 l; after second challenge = 0.32 l).

It is therefore suggested that repeated bronchoconstriction would be likely to result from chronic exposure to these dusts and that the constriction would be greater with pre-harvest retted flax.

We are indebted to the Eastern Health and Social Services Board for supporting this research, to the Lambeg Industrial Research Association for use of their facilities, and to Michael McGrath, Mervyn Murdock, and Nelson Ruddel for their technical help.

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