ASSESSMENT OF THE RISK OF INTRODUCTION OF EXOTIC FOREST INSECTS AND DISEASES WITH IMPORTED TENTS

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ABSTRACT

The risk of introduction of exotic insects and diseases with imported tents was assessed by examining 45 tents accompanying incoming passengers at the Auckland International Airport from 3 to 9 December, 1981. After brushing the tent free of gross debris (which is the normal quarantine procedure), eight 133 cm² areas of each tent roof were swept for 2 minutes with a filter holder containing a membrane filter attached to a vacuum pump. The filters, which retained all detachable material greater than 0.2 μm in diameter, were examined under the microscope. The filters from only 5 tents had more than 10% of their surface covered by the material removed from the tent roof, indicating that the majority of the tents examined were quite clean externally. The density of fungal spores on the tent fabric was close to that expected in outdoor air, showing that a deposit of spores had not accumulated on the tent roof. No spores of pathogens of forest trees were seen on the filters. It is concluded that the risk of introduction of diseases through spores carried on tent roof fabric is small. Potentially pathogenic fungi were, however, present on the debris collected from the tents and live insects were also found inside tents. This debris does pose a risk but the present practice of cleaning tents on arrival should reduce this risk considerably.

INTRODUCTION

Strict quarantine measures are the first line of defence against the introduction of exotic insects and diseases, especially in isolated, insular countries such as New Zealand, where the risk of such undesirable introductions by natural means is small. Because of the heavy dependence of New Zealand on agriculture, there is tight control on the import of animal and plant products (The Introduction and Quarantine of Plants Regulations 1973

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and The Forest Produce Import and Export Regulations 1966) which are subject to examination to make sure that they are free from pests and diseases before they are permitted entry. Other items likely to carry pathogenic organisms, such as footwear of travellers, are also examined and disinfected if necessary.

New Zealand is deservedly a popular country with those who have a taste for the outdoors and many such visitors bring camping equipment, including tents, with them. From February 1980 to January 1981, 1034 tents were brought in by incoming passengers at Auckland International Airport (Agriculture Quarantine Service records, Auckland). Many camping grounds particularly in North America, are situated in forests where major forest pathogens, disseminated by airborne spores, are present. Tents used near infected trees could become contaminated with spores of such pathogenic fungi and introduce the organisms to New Zealand if used in forested areas in this country. At present tents are brushed free of gross debris before they are allowed entry but this treatment would not remove spores, if they are present in or on the fabric. Imported tents thus seemed to be a potential source of introduction of airborne diseases (Sweet, 1975). The work reported here was therefore to see if any risk does exist. It was carried out with considerable help from officers of the Agriculture Quarantine Service.

MATERIAL AND METHODS

Selection of Tents

The study was restricted to passengers arriving at the Auckland International Airport as the majority of overseas flights (and all flights from countries other than Australia) terminate there. Agriculture Quarantine Service records showed that December is the most popular month for intending campers to come to New Zealand and the first week of December 1981 (3 December-9 December) was chosen for the study. All tents carried on 29 flights arriving over this 7-day period, except those used solely in Australia, were picked up by the examining Agriculture Quarantine Officers, who recorded the country or countries in which the tents were used and delivered them to the sampling area. Tents used solely in Australia were not examined as airborne spores are frequently carried across the Tasman by wind and it was considered that importation of tents was not likely to increase significantly the risk of introduction of diseases from Australia.

Sampling

Each tent was numbered and visually examined. The presence of soil and other debris was noted and it was then brushed clean. Any plant material or insects (but not soil) found in or on it were kept for laboratory examination. Eight 133 cm^2 portions of the tent roof (or fly, if present), 4 near the top and 4 near the bottom, were then delimited by affixing embroidery frames to the fabric. The frames also stretched the material tight and made sampling easier.

The delimited areas were swept for 2 minutes with a nozzle fixed to a membrane filter holder containing a filter (Millipore, 0.2 μm, 47 mm diam.) attached to a vacuum pump (93.32 kPa vacuum). Preliminary work in the laboratory, using *Penicillium* spores on a nylon tent had shown that in these conditions, 82% ($\pm$ 6%) of the spore load was removed. The filters (8 per tent) were individually numbered and stored in petri dishes.

Laboratory Examination

In the laboratory, two 2.5 cm^2 circles were punched from each filter. One of the circles was stained with aqueous aniline blue, dried, cleared in immersion oil, and examined under the microscope. The following observations and measurements were made on this circle:

(1) The percentage of the surface area of the filter covered by the deposited material was measured on 5 randomly chosen microscope fields using an Optomax Image Analyser.

(2) The fungal spore types and pollen grains were identified as far as possible.

(3) The number of hyphal fragments and fungal spores were counted in 5 randomly chosen microscope fields per circle. From these counts, the number per filter was calculated, which gave an estimate of the catch from one sampling frame (133 cm^2) of the tent.

(4) The abundance of organic and inorganic debris was estimated on a 0-3 scale (0 = absent, 1 = scarce, 2 = medium, 3 = abundant).

The second circle punched from the filter was placed on 0.1% malt agar for 72 hours to allow any viable spores that may have been present to germinate before staining, clearing and microscopic examination to determine the type and abundance of viable fungal spores.

The plant material recovered from the tents was incubated in damp chambers for 48 hours and examined under a microscope. The insects were passed on to an entomologist for identification.

RESULTS AND DISCUSSION

Forty-five tents were sampled over the 7-day period. Two of these tents (nos. 17 and 39) were coated with some material which became so thickly deposited on the filters that microscopical examination was not possible. The results therefore apply to a sample of 43 tents, 17 of which were last used in the U.S.A., 6 in Canada, 15 in continental Europe and 5 in the U.K. The results from all 8 filters collected from one tent were pooled together as frame position did not appear to affect any of the characters measured or assessed. A summary of the results for individual tents is given in Appendix 1.

The mean percentage of the surface area of the filters covered by a deposit indicated the amount of detachable material on the tent surface and hence generally the cleanliness of the tent (Table 1). Most of the tents were tolerably clean; the set of filters with more than 50% of the surface area covered in debris came from a tent which had become mouldly and most of the debris consisted of fungal hyphae. Four sets of filters had more than 10% but less than 50% cover; two of these had mainly organic debris, one inorganic debris (mostly sand grains) and one had a mixture of organic debris and sand grains.

TABLE 1: MEAN PERCENTAGE OF SURFACE AREA OF FILTERS COVERED BY DEPOSIT

Percent Cover	*Number of Tents*
1-10	38
11-50	4
>50	1

Microscopic examination of all the filters showed that single-celled, spherical, hyaline spores were most common. Other spore types present were basidiospores of Agarics, Polypores, and Gasteromycetes; dark, muriform, *Pleospora*-type spores; and dark, transversely-septate *Drechslera*-type spores. Ascospores of *Chaetomium* and conidia of *Cladosporium* and *Pithomyces* were also seen. One filter from tent 36 had four clusters of teliospores of *Puccinia pruni-spinosae* but no other spores resembling rust uredospores or teliospores were seen. No spores which could be

identified as propagules of known forest tree pathogens were observed. Circles of filters placed on 0.1% malt agar had colonies of Mucoraceous fungi or those of *Penicillium* spp.; spores of these saprophytes are very common in the air. The germination percentage of the spores present on the filters was never more than 20%. It would appear that most of the spores seen were not viable. Pollen grains were found on four tents and among the pollen grains present were those of *Quercus, Myrica,* and *Picea* spp.

The estimates of hyphal fragment and fungal spore numbers are given in Table 2. Over half of the tents (22 out of 43) had less than 1 spore or hyphal fragment per square centimetre and an additional 7 had less than 1 hyphal fragment/cm² and 6 had less than 1 fungal spore/cm². Only 1 tent had more than 5 hyphal fragments and spores/cm²; there was 1 tent with more than 5 hyphal fragments/cm² and 3 with more than 5 spores/cm². These tents may have been packed while still wet because they had a surface growth of mould and the deposited material contained large numbers of hyphal fragments and spores of the sooty mould type. These high numbers therefore have no plant pathological significance. The concentration of outdoor air spora varies with time, season, place, weather and human activity but the mean figure of 12 500 spores/m³ reported by Gregory (1973), from a continuous record over six months, may be taken as a guide. This spore load if uniformly deposited on a flat surface would give 1.25 spores/cm². The spore load of most tents is compatible with this estimate and the composition of the spora given by Gregory (1973)—*Cladosporium* conidia, hyaline and

TABLE 2: DENSITY OF HYPHAL FRAGMENTS AND FUNGAL SPORES ON TENT SURFACES (MEAN OF 8 SAMPLING FRAMES PER TENT)

No./cm²	*Hyphal Fragments*		*Fungal Spores*	
	Tent Nos.	*Total Tents*	*Tent Nos.*	*Total Tents*
0-1	1, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 26, 29, 30, 31, 32, 36, 37, 38, 40, 42, 45	29	1, 2, 3, 5, 9, 11, 12, 13, 14, 15, 18, 19, 20, 21, 22, 23, 26, 27, 28, 29, 31, 32, 33, 34, 38, 40, 42, 45	28
1.1-2	2, 4, 33, 34, 41, 44	6	6, 7, 8, 16, 41	5
2.1-3	27	1	4, 10, 35, 37	4
3.1-4	35	1	36	1
4.1-5	10, 24, 28, 43	4	44	1
>5	3, 25	2	24, 25, 30, 43	4

coloured basidiospores, dark, septate conidia of *Alternaria* and pollen grains—is also in general agreement with the types of spores found on the tents. It seems that the tents sampled had about the same concentration and composition of spores as would be expected in the air and, contrary to expectation, they did not accumulate a deposit of spores.

The assessments of the abundance of organic and inorganic debris are given in Table 3. Amorphous organic debris (mostly plant remains in various stages of decomposition), scales from butterfly wings and parts of insect legs were seen on the filters from most tents. Inorganic debris, most probably sand grains, was also frequently seen. The incidence of such materials probably reflects the nature of the camp sites where the tent was used.

TABLE 3: ASSESSMENT OF THE ABUNDANCE OF ORGANIC AND INORGANIC DEBRIS ON SAMPLE FILTERS (MEAN OF 8 SAMPLES PER TENT)

Rating	*Organic Debris*		*Inorganic Debris*	
	Tent Nos.	*Total Tents*	*Tent Nos.*	*Total Tents*
0 : Absent		0	12, 13, 19, 24, 25, 33, 34, 36, 37, 38, 42	11
1 : Scarce	3, 4, 5, 9, 11, 12, 14, 19, 24, 25, 26, 28, 30, 31, 32, 33, 34, 37, 38, 40, 45	21	2, 4, 5, 6, 8, 10, 11, 14, 15, 16, 18, 21, 23, 26, 27, 28, 30, 32, 35, 40, 44, 45	22
2 : Medium	1, 6, 8, 10, 13, 15, 16, 23, 29, 35, 36, 41, 42	13	1, 3, 7, 9, 20, 22, 29, 41	8
3 : Abundant	2, 7, 18, 20, 21, 22, 27, 43, 44	9	31, 43	2

The sweepings from the tents consisted mostly of grass stems and leaves, leaves of broadleaved plants, and conifer needles. A species of *Marssonina* (most probably pathogenic) was seen on poplar leaves collected from one tent and *Lophodermium baculiferum* (possibly pathogenic) was present on pine needles from another. The most common fungi seen were saprophytes such as *Epicoccum, Alternaria,* and *Pestalotia.* Live insects (all innocuous) were found in 6 tents. One tent yielded ants (*Iridomyrmex* sp.) and psyllids; insects found in the other tents were: *Propylea* (Coccinellidae) sp., *Thanasimus* (Cleridae) sp., and various members of Silphidae, Staphylinidae, and Tenebrionidae. The MAF interception lists show that insects are frequently found

in imported tents (Ministry of Agriculture and Fisheries, 1979, 1981).

CONCLUSIONS

The study showed that none of the suction samples taken from the 43 tents examined contained recognisable spores of known forest pathogens. However, the limits of the sampling and examination methods must be taken into account in evaluating this find. Soil accompanying tents was not examined, the sampling area covered only a limited area of the tent roof (1.6% of a normal 2-man tent), the sampling method employed did not remove all the spore load and sampled only the airborne spora and the filters were examined by only one observer who cannot have recognised all spores that were present. Therefore, it would not be correct to assume that no spores at all of forest pathogens were present either in the suction samples or on the tent surfaces. However, it can be safely assumed that spores of pathogens did not occur in large numbers. As the spore load on the tents resembled that of outdoor air in temperate countries, it appears that either the nature of the fabric or the manner and conditions in which tents are used, or both, must militate against accumulation of spores in the tent fabric. The spores on the tents also had a low germination percentage; presumably exposure of the tent roof to the sun had killed many spores. The inability of tent roofs to accumulate layers of spores, coupled with the low viability of the retained spores and the near-absence or absence of spores of forest pathogens, all indicate that the risk of transmission of pathogens by means of airborne spores carried on tents is small. The gross plant and animal debris, the soil, and the insects carried in or on tents present the major risk. It may be advisable to spray the inside and outside of imported tents with an insecticide to make certain that all insects are killed.

It is possible that the tents examined were last used in the northern hemisphere summer (June-August) and had lost some of their spore load while in storage. It would be necessary to examine tents arriving in New Zealand in our winter months to determine whether the microflora of recently used tents differs from those examined in this study.

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APPENDIX 1

Observations, Measurements, and Assessments made on Individual Tents

Tent No.	*Used in Country/Area*	*% Cover*	*Hyphal Fragments**	*Fungal Spores**	*Organic Debris†*	*Inorganic Debris†*	*Gross Vegetable Matter*	*Insects*	*Remarks*
1	USA/Grand Canyon	3	15	120	2	2	None	None	
2	UK	8	170	125	3	1	Grass	None	
3	Canada	59	1000	95	1	2	None	None	Tent mouldy
4	Canada	2	205	450	1	1	None	None	
5	UK/New Forest	1	75	95	1	1	None	None	*Quercus* pollen
6	USA/Idaho	2	45	210	2	1	None	None	Pollen
7	USA/Arizona	8	115	165	3	2	Needles	None	
8	USA/Colorado	5	50	210	2	1	Needles	None	
9	UK	1	55	75	1	2	None	*Propylea* sp.	
10	Germany	20	595	355	2	1	Grass	None	Tent mouldy
11	Switzerland	1	25	15	1	1	None	None	
12	Switzerland	2	10	10	1	0	None	None	
13	Netherlands	1	10	0	2	0	None	None	
14	Netherlands	2	40	85	1	1	None	None	
15	Switzerland	4	105	50	2	1	None	None	*Myrica* pollen
16	Switzerland	5	130	145	2	1	None	None	
17	USA, UK, Spain	—	—	—	—	—	Grass leaves	None	*Epicoccum*
18	USA/Hawaii	3	50	60	3	1	None	None	
19	USA/Hawaii	1	0	0	1	0	Grass leaves	None	
20	Canada	8	80	95	3	2	Needles	None	
21	USA/Utah, Ariz., Calif.	2	75	95	3	1	Leaves	None	*Alternaria*
22	USA	13	110	60	3	2	None	*Iridomyrmex*, psyllids	
23	USA/Colorado	4	70	50	2	1	None	None	
24	UK	6	635	925	1	0	None	None	Tent mouldy

25	Canada	4	1020	840	1	0	Needles, leaves	None	Tent mouldy
26	USA/Nevada	4	15	10	1	1	None	None	
27	USA	18	380	75	3	1	Needles	None	
28	Canada	5	650	70	1	1	None	None	
29	Canada	3	100	25	2	2	None	None	
30	Europe	10	15	850	1	1	None	None	Tent mouldy
31	USA/Virginia	31	25	30	1	3	None	None	
32	UK	4	10	10	1	1	None	None	
33	USA/California	4	220	130	1	0	Needles	None	
34	Netherlands	1	255	85	1	0	None	None	*Picea* pollen
35	Germany	7	415	395	2	1	Twigs	Silphidae	
36	Italy	3	90	400	2	0	Needles	None	*Puccinia pruni-spinosae*, *Lophodermium baculiferum*
37	Europe	3	30	270	1	0	Willow, poplar	*Thanasimus*	*Marssonina*
38	UK	2	50	60	1	0	None	None	
39	Germany	—	—	—	—	—	None	None	
40	Europe	4	30	120	1	1	None	Staphylinidae	
41	USA	6	135	170	2	3	Grass leaves	None	*Alternaria*
42	Europe	7	65	40	2	0	Leaves	None	
43	USA/California	6	585	695	3	3	None	Tenebrionidae	Tent mouldy
44	Europe	5	135	600	3	1	None	None	Tent mouldy
45	USA	1	30	85	1	1	Needles	None	*Ceuthospora*

*Number per sampling frame of 133 cm² (mean of 8 filters), extrapolated from counts made on 5 microscope fields per filter and rounded off to the nearest 5.

†Mean of assessments made on 8 filters on a 0-3 scale and rounded off to the nearest whole number.