Preliminary observations on black mould (*Aspergillus niger*) on onions in the Pukekohe area

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Report to the New Zealand Onion Exporters' Association

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CONTENTS

EXECUTIVE SUMMARY

Preliminary observations on black mould (*Aspergillus niger*) on onions in the Pukekohe area

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HortResearch was commissioned by the Onion Exporters Association of New Zealand to make a preliminary study of black mould in onions in the Pukekohe area. The study aimed to examine the relationship between thrips infestation, handling methods during harvest, and the development of black mould during storage. Observations were also made on the incidence of *A. niger* spores on onions in several commercial properties as the crops were approaching maturity.

In the storage trial, the key results were:

1. Contamination of mature bulbs by the black mould fungus was able to be detected by a swab method and by direct isolations from bulb tissue. Because of its economy and ease of use, of the swab method, which primarily detects spores, is recommended for routine use.
2. The fungus was detected more frequently on thrips damaged bulb tissue than on bulbs without thrips damage.
3. Black mould developed only in the line of onions that had the highest level of fungal contamination pre-storage. No mould developed in lines that had relatively lower levels of contamination.
4. There was a higher incidence of black mould in onions stored under simulated shipping conditions than those held at ambient New Zealand temperature.

Monitoring of *A. niger* spore levels in commercial fields showed that there were insignificant levels of spore contamination in all fields sampled.

Although these results can be regarded as little more than preliminary observations on the disease, they suggest a number of lines of investigation that may lead to improved disease management methods.

1. The relationship between thrips incidence and presence of black mould contamination needs further investigation. While thrips injury may be one factor predisposing onions to storage mould, the current experiment was unable to separate thrips injury from other site related factors.
2. Possible relationships between crop handling methods at harvest (standing topping, hand topping, topping after curing etc) and levels of fungal infestation need to be further investigated.
3. An audit trail, which would enable heavily diseased lines in export consignments to be traced back to the field of origin, should be established. Such information could be used to help identify factors contributing to the problem and ultimately allow growers and exporters to assess the potential risk from black mould in any particular crop.

4. The relationship between levels of *A. niger* contamination on bulbs and development of mould in storage needs to be confirmed. If a consistent, quantitative relationship can be established it could be used to develop a pre-export assessment system to predict the likely incidence of black mould at outturn on overseas markets.

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INTRODUCTION

New Zealand exports over 200,000 tonnes of onions per annum, mainly to the United Kingdom, Europe and Japan. Over the past two seasons, problems have been experienced with a high incidence of black mould (*Aspergillus niger)* in consignments arriving in export markets. The disorder depresses the market value and, in some markets (e.g. Germany), can lead to affected consignments being destroyed, resulting in severe losses for exporters.

Black mould is caused by *Aspergillus niger,* a fungus commonly found on dead vegetable material. It is a "high temperature" fungus, thriving at temperatures over 30°C. It is well known to cause black mould of onions in tropical areas, as well as in temperate areas where onions are artificially dried at temperatures over 30°C. While it has often been recorded on onions in New Zealand, it is only over the past few seasons that damage has been on a scale to cause economic loss and market access problems. Little is known of the disease in New Zealand.

In recent years many onion crops in the Pukekohe district have been affected by heavy infestations of onion thrips (*Thrips tabaci*) leading to high thrips populations and damage in bulbs of both stored and exported onions. There is a feeling amongst growers and exporters that the thrips and black mould problems are linked with thrips infestation predisposing bulbs to black mould. Different methods of field handling (mechanical topping of standing onions, hand clipping, topping after field curing) may affect both the severity of thrips infestation and incidence of black mould. Thrips numbers and black mould damage are thought to increase during shipping as the onions pass through the tropics *en route* to Europe.

The effect of different handling systems on thrips infestations in bulbs, and changes in thrips populations after harvest are being studied in a Technology for Business Growth programme funded by Technology New Zealand and the Onion Exporters Association of New Zealand (OEA). In association with that project an introductory study has been made of the relationship between thrips infestation, shipping conditions and incidence of black mould. The objectives of the trial were:

1. Determine the relative amount of black mould on onions at maturity.
2. Relate initial incidence of black mould to the amount developed under simulated shipping conditions.
3. Relate incidence of black mould after shipping to incidence of thrips in bulbs at harvest.

Observations were also made on the incidence of black mould on maturing bulbs in a number of commercial properties in the district.

MATERIAL AND METHODS

Storage trial

Onions were collected from three commercial properties known, from field observations, to have had different severities of thrips infestation and to have been handled in different ways. In February 2000, between 300 and 400 bulbs were collected from each property and divided into two equal batches. One batch was stored at ambient temperature; the other in a controlled environment room programmed to simulate temperatures and humidities prevailing during shipping to Europe. Details of the proposed and actual storage regime are provided in Appendix 1.

The characteristics of the different lines of onions and the storage treatments are shown below:

Treatment	Site	Thrips incidence	Topping	Storage
1a	Waiuku	Heavy	Machine topped after field curing	Ambient
1b	Waiuku	Heavy	Machine topped after field curing	Ship
2a	Karaka	Low	Handclipped	Ambient
2b	Karaka	Low	Handclipped	Ship
3a	Pukekawa	Low	Machine topped (Samon topper)	Ambient
3b	Pukekawa	Low	Machine topped (Samon topper)	Ship
4	Karaka	Low	Machine topped	Ambient

Onions in Treatments 1a and 1b were taken from the grower's shed; dates of harvest were not available. Onions in Treatments 2a and 2b were hand clipped in mid-January, and left in the field until collected for the trial on 23 February. Onions in Treatments 3a and 3b were topped in mid-January and collected for the trial on 21 February. Treatment 4 was included as a late addition to the protocol when, by coincidence, it was found that some of the onions in the hand clipped Karaka site (Treatments 3a, 3b) had been machine topped. Only 100 bulbs were collected and they were stored at ambient temperature only.

For treatments 1a-3b, 50 bulbs were individually numbered for laboratory assay for the presence of *A. niger*. For the high thrips line (Waiuku), the 50-bulb sample consisted of 25 bulbs selected for severe thrips injury and 25 bulbs with no visible thrips injury. For all other lines, a single, randomly selected 50-bulb sample was used.

Two methods were used to detect the fungus:

1. **Swab technique.** Swabs (sterile Cultiplast medical swabs) were prepared by removing the swab from the tube, dipping the cotton wool tip in sterile, molten Potato Dextrose Agar (PDA) containing the antibiotics Ampicillin and Rifampicin (to inhibit bacterial growth) and replacing them in their tubes. The dry scale leaves were peeled back approximately 1cm from the neck of the bulb and the exposed white storage leaf was swabbed by wiping with the agar-impregnated swab. When bulbs were infested by thrips, the swabs were taken from the thrips damaged areas.

2. **Tissue isolation.** Four pieces of tissue (approx. 4mm^3) were taken from the exposed storage leaf tissue previously used for taking the swab and placed on a petri dish of antibiotic amended PDA.

After assay, the 50 bulb sub-samples were placed in Netlon bags and returned to the bulk of the line for each treatment. The swabs and PDA plates were incubated at 35°C and examined for the presence of *A. niger* after 4 days.

The simulated shipping trial commenced on 22 March and the onions were removed on 25 April. Onions for ambient storage were held at approximately 23°C for the same period. At the end of the storage period, all onions were cut longitudinally and examined for the presence of black mould.

Field incidence of *A. niger*

Swabs were made from the lower leaves and above ground surfaces of bulbs on various commercial properties. The properties were those on which HortResearch white rot trials were being run, or were being used for thrips monitoring in the TBG programme. Ten swabs were taken from individual onions in each property at each date, incubated at 35°C, and examined for the presence of *A. niger*.

RESULTS AND DISCUSSION

Storage trial

Pre-storage assessments.

The results of the pre-storage black mould assessments are shown in Table 1.

Table 1. Recovery of *A. niger* and other fungi from different lines of onions by swabbing and plate isolation prior to storage.

	Swabs			Plate isolations				
Trt	No of bulbs swabbed	No. with *A. niger*	% *A. niger*	No of bulbs isolated	No. with *A. niger*	% *A. niger*	% *Penicillium* sp.	% *Rhizopus stolonifer*
1a	25 no thrips visible	2	8	25 no thrips visible	2	8	42	4
	25 severe thrips	12	48	25 severe thrips	3	12		
1b	50	22	44	20[1]	6	30	40	10
2a	50	8	16	20	2	10	80	0
2b	50	2	4	20	1	5	70	0
3a	50	0	0	20	1	5	90	0
3b	50	0	0	20	2	10	75	15

[1] Taken from the sample of 50 used for swabbing.

Both techniques detected *A. niger* contamination of bulbs. In the heavily thrips infested Waiuku line, there was greater recovery of the fungus from bulbs which were heavily infested by thrips. Hand-clipped onions from the Karaka site, known to have low thrips numbers prior to harvest, were found to have a high proportion of thrips infested bulbs when assayed. This is consistent with observations that thrips invaded bulbs after hand clipping (Andrew Tomkins pers. com.). The incidence of *A. niger* contamination in the Karaka line was much less than in the Waiuku line despite the moderately high thrips infestation. Bulbs from the Pukekawa site had relatively few thrips and that is reflected in the lower recovery of the fungus from those onions.

The fungi most frequently found in tissue isolations from all samples were *Rhizopus stolonifer* and varieous species of *Penicillium.* Based on cultural characteristics, at least three species of *Penicillium* were present. All were high temperature species; i.e. they were able to grow at 35°C, a temperature at which most temperate fungi will not grow.

Post-storage assessments

The incidence of black mould and bacterial soft rot in bulbs after storage are shown in Table 2. For ease of comparison the incidence of black mould in pre-storage assessments is also shown.

Table 2. Incidence of *A. niger* infestation of bulbs prior to storage and incidence of black mould and bacterial soft rot symptoms after storage.

	Pre-storage		Post-storage		
	Swab	Plate			
Trt	*% A. niger*	*% A. niger*	No. onions[2]	% Black mould	Bacterial soft rot
1	28	10	250	0.8	6.8
2	44	30	250	2.4	9.6
3	16	10	200	0	0
4	4	5	200	0	1
5	0	5	225	0	0
6	0	10	250	0	0
7			100	0	0

[2] Includes the sample of 50 onions used for prestorage assessment in each treatment

Black mould and bacterial soft rot was found only in onions from the Waiuku site. In almost all cases soft rot was associated with bulbs that had germinated during storage. The development of black mould in stored onions from the Waiuku site correlates broadly with the higher incidence of recovery of the fungus from bulbs in pre-storage assessments. The greater incidence of the disease in the "shipped" onions than those stored at ambient temperature suggests that the simulated shipping conditions did promote the development of the disease. While the line of onions with the highest thrips infestation also developed black mould, there is insufficient data from the trial to separate the effects of thrips from other site effects in the development of disease. The lack of mould development in onions from the Karaka and Pukekawa sites prevented any comparison of effects of hand or machine clipping.

Field incidence of *A. niger*

The properties, sample dates and recovery of A. *niger* are shown in Table 3.

Table 3. Recovery of *A. niger* (number of recoveries/number of swabs) from commercial onion properties, Pukekohe 1999-2000.

No.	Property	Sample dates							
		15 Dec	22 Dec	30 Dec	7 Jan	12 Jan	18 Jan	3 Feb	8 Feb
1	Tuakau	0/10			1/10				
2	Patumahoe	0/10	1/10	1/20	0/20				
3[1]	Pukekohe					1/10			
4	Pukekawa					0/10	0/10		
5	Karaka					0/10	0/10		
6	Waiuku					0/10	0/10	0/9	0/9
7	Bombay 1					0/10	0/10	0/10	0/10
8	Bombay 2					0/10	0/10	0/10	0/10

[1] Properties 3-8 are those used in the OEA thrips monitoring programme. The Waiuku property used for these observations was different from that used for the storage/shipping trial

A. niger was rarely detected in the commercial fields. The detection method (swabs) used in the field sites was shown in the preharvest bulb assays to be relatively efficient at demonstrating the presence of the fungus. Thus it is concluded that there was very little spore contamination of bulbs and foliage as those fields approached maturity.

This series of observations represents the first attempt to obtain an understanding of the black mould problem in New Zealand. Several key points have emerged:

1. Both the swab method and direct isolations from bulb tissue were able to detect contamination of mature bulbs by the black mould fungus. Although tissue isolations appeared to be marginally more efficient than swabs when there is a very low incidence of the fungus, the ease and economy of the swab method (that primarily detects spore contamination of bulbs) would make it the preferred method for routine assessments.
2. The fungus was detected more frequently on thrips damaged bulb tissue than on bulbs without thrips damage.
3. Black mould developed only in the line of onions that had the highest level of fungal contamination pre-storage. No mould developed in other lines which had relatively lower levels of contamination.
4. There was an indication that the incidence of black mould was increased by shipping conditions.
5. All of the bulbs sampled in commercial crops in the weeks leading up to harvest had insignificant levels of spore contamination.

Although these results can be regarded as little more than preliminary observations on the disease, they suggest a number of possible lines of investigation that may lead to better disease management methods.

1. The relationship between thrips incidence and presence of black mould contamination needs further investigation. In one line of onions in the present study, heavy thrips infestation was associated with high rates of contamination by *A. niger* spores. In another line with moderate levels of thrips infestation, less spore contamination was found. While thrips injury may be one factor predisposing onions to storage mould, there are undoubtedly other site-related factors contributing to the problem.
2. The possible relationships between crop handling methods at harvest (standing topping, hand topping, topping after curing etc) and levels of fungal infestation need to be further investigated.
3. The establishment of an audit trail, allowing heavily diseased lines in export consignments to be traced back to the field of origin, would offer several important benefits. Used in association with routine crop inspections and health assessments, it would assist in identifying factors contributing to the problem, and would allow growers and exporters to assess the potential risk of black mould in any particular crop.
4. The relationship observed in the preliminary trial between levels of *A. niger* contamination on bulbs and development of mould in storage needs to be confirmed. If a consistent, quantitative relationship can be confirmed it may be possible to develop a pre-export assessment system which would predict the likely incidence of black mould at outturn on overseas markets.

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APPENDIX 1.

Proposed storage regime for simulated shipping voyage of onions to Europe and actual conditions in store (overleaf).

Basis for conditions:

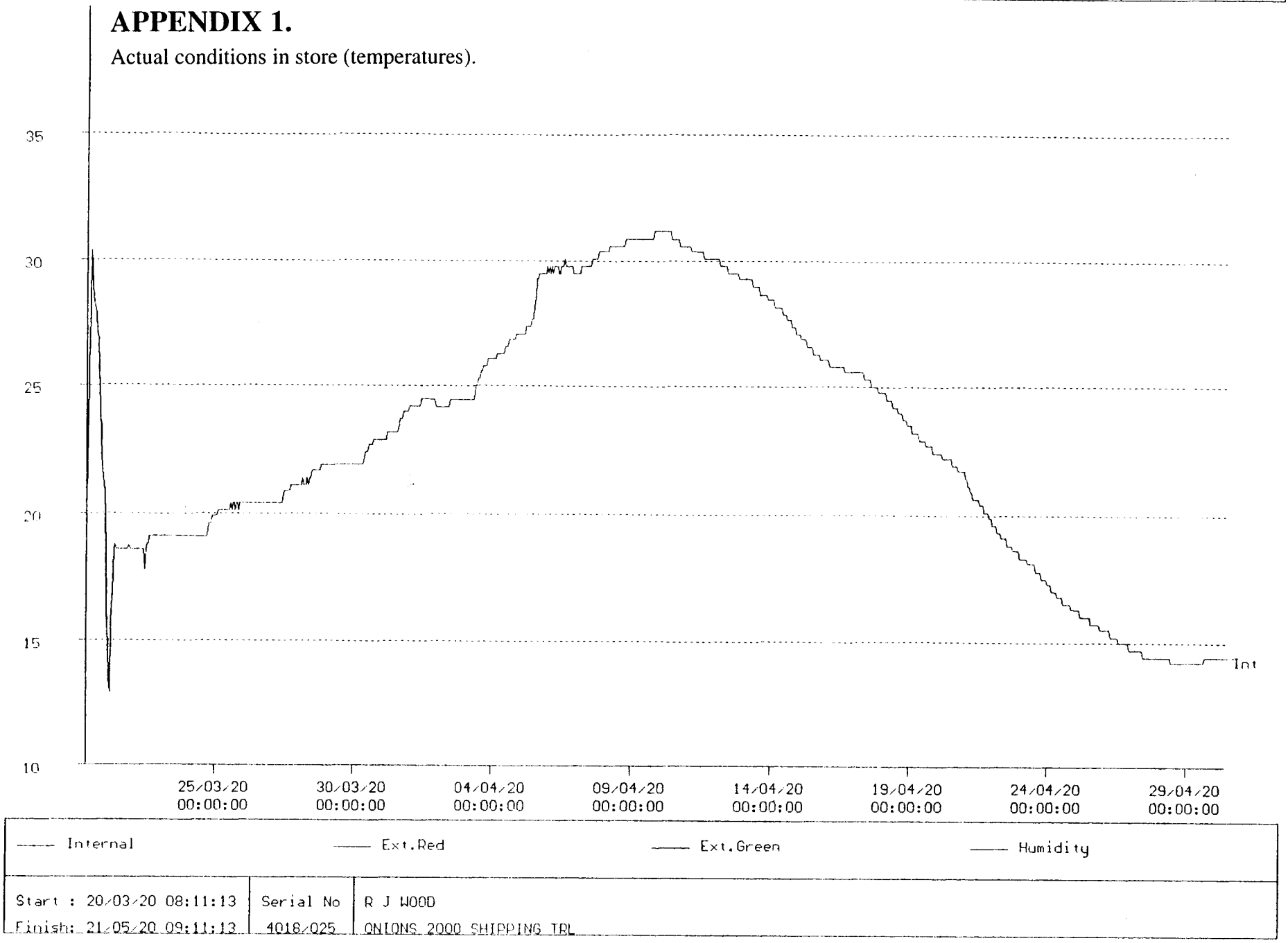
- 35 day journey via Panama canal.
- Apply slightly higher temperatures than ranges expected for voyages

Day	Temperature °C	Relative Humidity (%)
0	18-20 (ambient)	75
1-5	20-22	75
6-10	22-25	90-95
11-15	25-30	90-95
16-20	30-32	90-95
21-25	30-25	75
26-30	25-20	70
31-35	20-15	70

Programme provided by Richard J Wood, Vegcon Services Ltd., based on actual conditions recorded in transit.

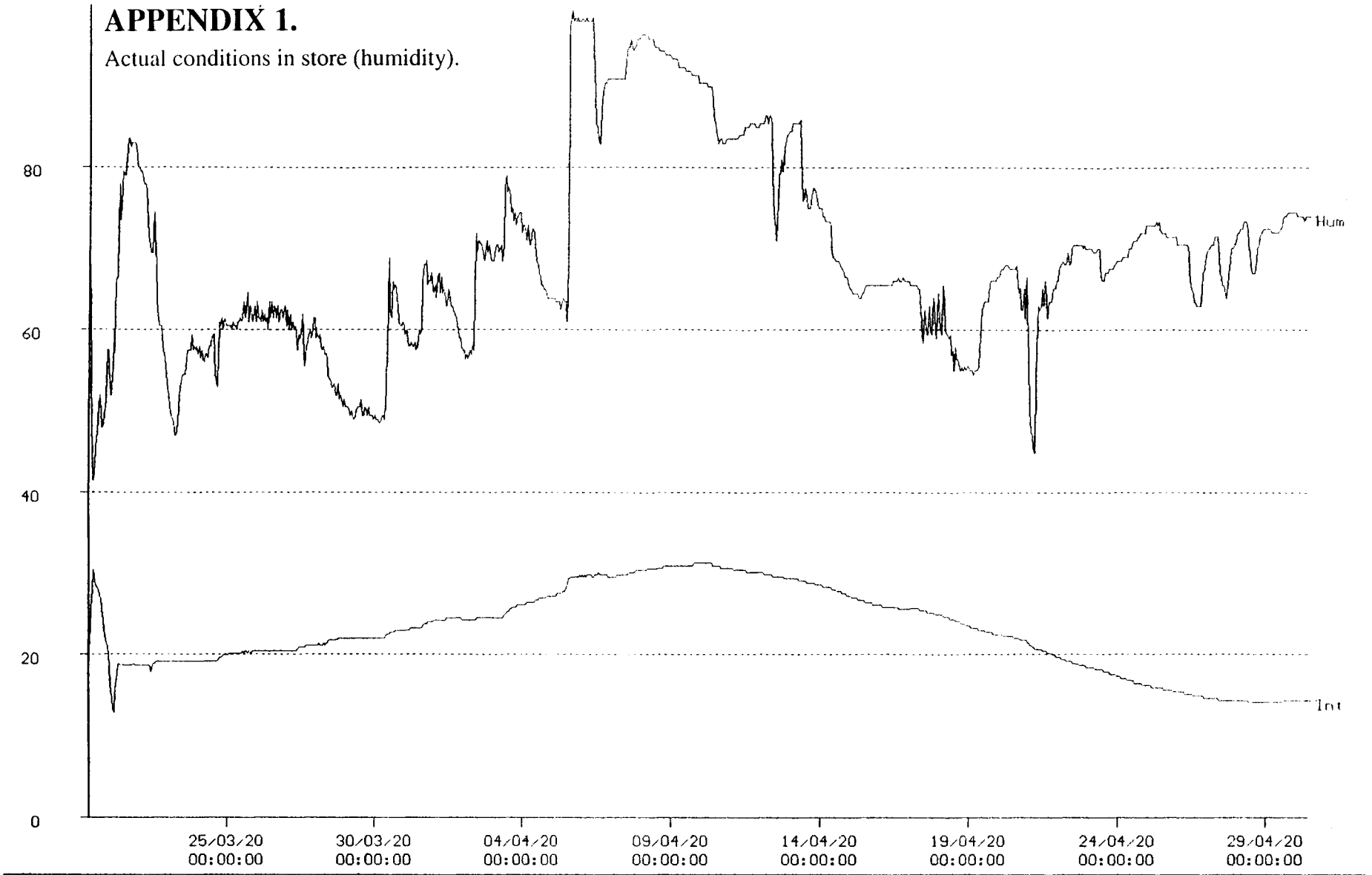
APPENDIX 1.

Actual conditions in store (temperatures).



APPENDIX 1.

Actual conditions in store (humidity).



Start : 20/03/20 08:11:13	Serial No	R J WOOD
Finish: 21/05/20 09:11:13	4018/025	ONIONS 2000 SHIPPING TRL