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# The Water Content/Water Activity Relationship of Cured Tobacco and Water Relations of Associated Spoilage Fungi

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*ABSTRACT*

*The relationship between moisture content and water activity ($a_w$) in cured tobacco was significantly influenced by sugar content. Overall, high sugar tobaccos such as Oriental and Virginia had a higher moisture content at any given water activity compared to low sugar tobaccos such as Burley. Virginia and Burley were both predominantly colonised by* Aspergillus *and* Penicillium *spp. Of these, about 80% of isolates could germinate at between 0·75 and 0·85 $a_w$, equivalent to moisture contents of between 18% and 24% in Burley and between 22% and 31% in Virginia. Growth of the dominant* Aspergillus *and* Penicillium *spp. was much slower on Virginia and Burley tobacco extract than on malt extract agars over the range 0·85 to 0·98 $a_w$. For some species the optimum $a_w$ for growth on tobacco extract medium was altered from that on the richer malt extract agar and for some there was also a significant difference in growth between Virginia and Burley extract agars. The mould-free storage periods for five different tobacco types was influenced by $a_w$. Visible moulding occurred within 7–14 days at 0·85–0·90 $a_w$ but only after about six months at 0·70–0·75 $a_w$. There were some differences in rate of moulding between tobacco types as well as in the range of fungi isolated at different $a_w$ storage levels.*

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## INTRODUCTION

In tobacco, moisture content is important in handling, manufacture and storage. For example, 30% moisture is required to keep tobacco soft and pliable for safe handling (Jeffrey, 1940), whilst during the manufacturing process moisture contents ranging between 12% and 30% may be necessary at different stages depending on the final product (Welty & Stout, 1970; Mitchell & Stauber, 1981). For the tobacco grower, moisture directly affects gross return (Welty & Nelson, 1971), and it is therefore often necessary for the manufacturer to re-dry the tobacco to a moisture content between 11% and 15% (wet weight basis). The industry regards this range as the level below which the risk of fungal attack is greatly reduced (Lucas, 1975).

The moisture holding capacity of tobacco may be further influenced by cultural practices which alter the leaf's physical and chemical composition, as well as by post-harvest treatment and curing methods (Wolf, 1967; Akhurst, 1971). Differences in the hydroscopicity may be observed not only between different tobacco types and varieties, but often within different grades of the same variety. For example, Bacot (1960) has demonstrated differences in flue-cured tobacco of the same grade and quality, where lighter coloured grades were more hydroscopic than darker coloured grades. Similar differences have also been reported in Maryland tobacco from three different crop years (McKee, *et al.*, 1984).

Total moisture content does not, however, give a good indication of the amount of water available to support microbial growth. This is more accurately described by the water activity ($a_w$) of the substrate (Scott, 1957). Water activity plays an important role in the growth and development of fungi, and different levels may be critical for germination, linear growth and sporulation. Maximum, minimum and optimum water activities at different stages of growth are significantly influenced by interactions with environmental factors such as temperature, pH and nutrient availability (Ayerst, 1969; Pitt & Hocking, 1977; Magan & Lacey, 1984*a,b*). At present, very little detailed information is available on the relationship between the moisture content, $a_w$ and associated fungi in flue-cured tobaccos. This may be critical in determining the safe storage life for different tobacco blends.

Studies by Welty *et al.* (1968) demonstrated that 'field fungi' such as *Alternaria, Cladosporium, Epicoccum, Nigrospora* and *Trichoderma* were predominantly isolated from tobacco before and immediately after curing. However, in stored tobacco, *Aspergillus* and *Penicillium* spp. predominated (Welty & Lucas, 1969) and accounted for at least 71% of fungi recovered (Welty & Lucas, 1968). Other prevalent fungi included

species of *Alternaria, Chaetomium, Rhizopus, Fusarium, Nigrospora* and *Syncephalastrum* (Welty & Lucas, 1969; Welty, 1972; Lucas, 1975). The groups of spoilage fungi which dominate stored tobacco at different $a_w$ levels have not previously been determined. Furthermore, very little information is available on the minimum $a_w$ levels permitting germination and growth of these spoilage fungi isolated from stored tobacco. Such studies would provide a better understanding of their deteriogenic potential and allow better prediction of the range of moisture contents over which safe storage would be permitted for different tobacco blends.

The aims of this investigation were (1) to determine the moisture sorption isotherms of a range of cured tobacco blends, (2) to determine the effect of $a_w$ on tobacco storage life and fungal colonisation, and (3) to assess the effect of $a_w$ on germination and growth of spoilage fungi in pure culture studies.

## MATERIALS AND METHODS

### Moisture sorption isotherms

Air-cured Burley, flue-cured Virginia, flue-cured Indian, flue-cured Malawi and Oriental tobaccos were preconditioned to moisture contents between 15% and 30% (wet weight), by the addition of water according to the following relationship

$$\text{added water (cm}^3\text{)} = (\text{tobacco dry weight} \times 100)/100 - \text{required M.C} - \text{sample weight}$$

The samples were stored at 4°C for 24–28 h, with frequent mixing, to allow equilibration to occur.

Water activity was then determined in five subsamples (1–2 g each), at 25°C, using the Humidat IC II (Novasina AG, Switzerland). Measurements were determined to an accuracy of within 0·002 units. Sorption isotherms were then constructed by plotting moisture content against water activity. The chemical composition of the different tobaccos was also determined independently by Rothmans International.

### Isolation methods

Fungi were isolated from Burley and Virginia tobaccos, which were representative of high- and low-sugar types, using both serial dilution and direct plating methods.

Serial dilution involved suspending 10 g finely chopped subsamples of each tobacco type in 90 ml of 0·1% sterile distilled water and homogenising four such samples in a Colworth Stomacher 400 for 10 min. A dilution series was prepared and 0·2 ml aliquots of appropriate dilutions were spread plated onto a range of agars.

Tobacco segments of 1–2 $cm^2$ were either directly plated or first washed in three successive changes of sterile water prior to plating onto selective agars (Welty & Lucas, 1968; Bell, 1971).

## Media

The media used were 3% malt extract (MEA), 3% malt 10% sodium chloride (MSA), and malt yeast extract with 40% glucose (MY40G). All plates were sealed with parafilm and incubated at 25°C for a maximum of three months.

## Water activity and growth of fungi

The ability of 106 isolates to germinate and grow at three specific water activities ($a_w$), 0·75, 0·80 and 0·85 $a_w$ was determined. This was carried out on 3% malt extract agar, modified with glycerol to the required $a_w$ (Scott, 1957) and incubated at 25°C for a maximum period of three months. Plates were microscopically examined for the onset of spore germination and growth which was considered to have occurred when at least five replicate plates had grown to at least 5-mm diameter.

More detailed studies of the dominant isolated fungi were made to determine the range of $a_w$ levels over which growth may occur. These experiments were carried out on 3% malt extract agar and tobacco extract agar modified with glycerol to 0·75, 0·80, 0·85, 0·90, 0·98 and 0·995 $a_w$. (Water availabilities of 0·85–0·90 are often present in processed tobaccos, particularly after the addition of additives and humectants). Tobacco extract was prepared by steaming 10 g $litre^{-1}$ ground tobacco in the glycerol solution for 30 min, after which they were removed by filtration, and agar added at 15 g $litre^{-1}$. In all cases the media were adjusted to pH 6 using 1M HCl.

Plates were inoculated using 2 $\mu$l aliquots of spore suspensions prepared in 0·1% distilled water agar, from seven-day-old cultures of *Aspergillus amstelodami, A.ruber* (both on MY40G), *A.niger, A.fumigatus, Penicillium chrysogenum* and *P.viridicatum* (all on MEA). Ten replicates were prepared at each $a_w$ level for each species and incubated at 30°C for up to three months. Tests were carried out at 30°C to simulate the working temperature often encountered in tropical climates. Plates of the

same $a_w$ were sealed in polyethylene bags. The colony diameters were measured and the growth rate in mm $day^{-1}$ calculated by linear regression analysis. Analysis of variance tables were then computed from regression equations and confidence limits calculated at the 99% level.

### Tobacco storage tests

Tobacco samples (30g) were conditioned to moisture contents equivalent to water activities ranging between $< 0{\cdot}70$ and $0{\cdot}90\ a_w$. They were then sealed into glass jars and stored at 30°C for up to 12 months. During this time, they were regularly inspected for visible mould growth, and results recorded as time taken prior to mould growth. All tobaccos which became mouldy were subsequently sampled, and the spoilage organisms identified.

## RESULTS

### Water sorption isotherms

The relationship between water activity and moisture content of each tobacco blend is shown in Fig. 1. With the exception of the moisture content range 17–19%, all five were found to have significantly different moisture contents at any given $a_w$. Overall, the moisture content range (equivalent to $0{\cdot}70$–$0{\cdot}90\ a_w$) for Virginia tobacco was highest, closely followed by Oriental. There was however little difference in the sorption isotherm positions of Burley, Indian and Malawi. The sugar and total nitrogen contents of the different tobaccos are shown in Table 1. Total sugar content was highest in Oriental and Virginia and lowest in Burley. The water sorption isotherms show that there is a significant difference between the high-sugar and low-sugar contents. There was little difference in total nitrogen of Virginia and Burley tobaccos.

### Fungal flora of tobacco and effect of water activity on growth

The range of species isolated from Virginia and Burley tobaccos is shown in Table 2. Quantitative assessment showed that *Aspergillus* spp. dominated over *Penicillium* spp. but their relative proportions were similar on both tobacco types (Table 3). *A.niger, A.amstelodami, A.ruber* and *P.chrysogenum* were the most frequently isolated species.

The minimum $a_w$ tolerated by a range of isolates of each species is

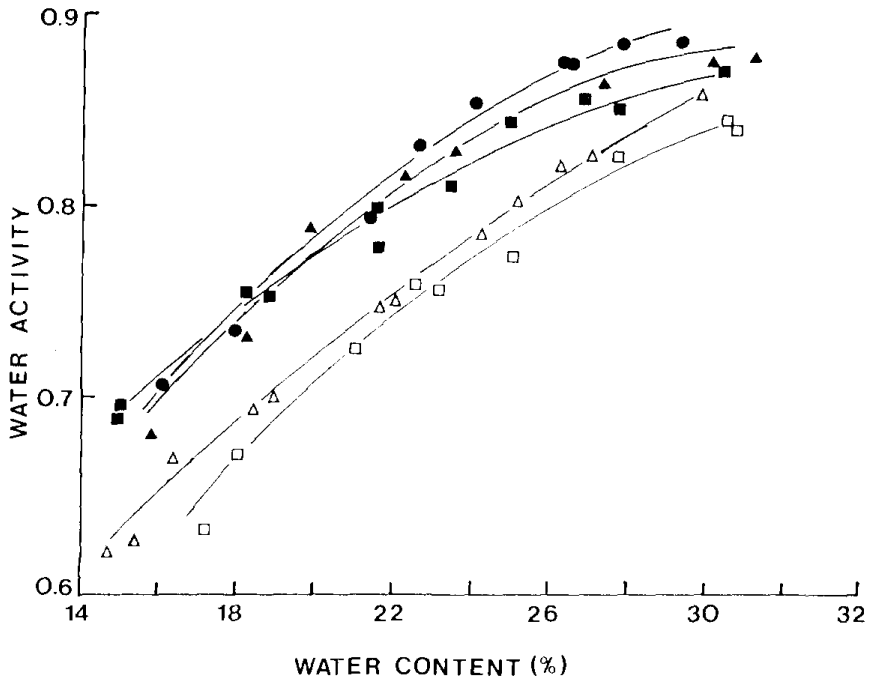


**Fig. 1.** Moisture sorption curves (adsorption) showing the relationship between water content (wet weight basis) and water activity for Burley (●) Virginia (□) Indian (■) Oriental (△) and Malawi (▲) flue-cured tobaccos at 25°C.

shown in Table 4. In general, of the 71 *Aspergillus* isolates examined, 11% grew down to 0·75 $a_w$, 49% at 0·80 $a_w$, and 27% at 0·85 $a_w$. Species of *A.amstelodami, A.niger, A.versicolor, A.chevalieri* and *A.flavus* were all found to include strains with different $a_w$ minima for germination and growth.

**TABLE 1**

The Total Sugar Content (% Dry Weight) of Burley, Virginia, Indian Flue-Cured, Malawi Flue-Cured and Oriental Tobaccos Including the Total Nitrogen in Burley and Virginia Tobaccos.

| | *(% Dry weight sugars)* | | | | |
|---|---|---|---|---|---|
| *Tobacco type* | *Fructose* | *Glucose* | *Sucrose* | *Total sugars* | *Total nitrogen* |
| Oriental | 7·7 | 8·0 | 1·7 | 17·4 | N.D. |
| Virginia | 6·8 | 5·5 | 0·8 | 13·1 | 2·4 |
| Indian | 4·7 | 2·9 | 0·9 | 8·5 | N.D. |
| Malawi | 3·8 | 2·0 | 0·6 | 6·4 | N.D. |
| Burley | < 0·5 | < 0·5 | < 0·5 | < 1·5 | 4·3 |

N.D., Not determined

**TABLE 2**
Comparison of the Range of Genera and Species Isolated from Burley and Virginia Tobaccos.

| *Organism* | *Tobacco type* | |
|---|---|---|
| | *Burley* | *Virginia* |
| *Aspergillus amstelodami* | + | + |
| *A.chevalieri* | − | + |
| *A.repens* | + | + |
| *A.ruber* | + | + |
| *A.versicolor* | + | − |
| *A.sydowii* | + | − |
| *A.niger* | + | + |
| *A.awamori* | − | + |
| *A.fumigatus* | − | + |
| *A.flavus* | − | + |
| *Penicillium chrysoenum* | + | + |
| *P.viridicatum* | + | − |
| *P.crustosum* | − | − |
| *P.spinulosum* | − | − |
| *P.restrictum* | + | − |
| *P.simplicissimum* | − | + |
| *Chaetomium globosum* | + | + |
| *Rhizopus* sp. | + | + |
| *Trichoderma* sp. | + | − |
| *Syncephalastrum racemosum* | − | + |
| *Cladosporium* sp. | + | + |
| *Mucor* sp. | + | + |

+, Present; −, not present.

Of the 22 *Penicillium* species none grew at 0·75 $a_w$, 27% grew at 0·80 $a_w$, 64% at 0·85 $a_w$ and 9% were unable to germinate at 0·85 $a_w$. Only *P.chrysogenum* was found to include isolates which varied in their minimum $a_w$ for growth. Of the other fungi examined, spores of *Scopulariopsis brevicaulis* and *Syncephalastrum racemosum* were only able to germinate at 0·85 $a_w$.

In these tests it was found that the time before onset of germination and growth was increased by reducing substrate $a_w$, ranging from about one week at 0·85 $a_w$ to about six weeks at 0·75 $a_w$. For example, in the *Aspergillus glaucus* spp. growth occurred within four days at 0·85 $a_w$, compared to between 7 and 19 days at 0·80 $a_w$ and 32–42 days at 0·75 $a_w$. Similarly, isolates of *A.versicolor* and *A.niger* grew within 3–6 days at

**TABLE 3**
Mean Numbers of Colony Forming Units (CFU) ($g^{-1}$ Fresh Weight) of *Aspergillus, Penicillium* and Other Fungi Isolated from Burley and Virginia Tobaccos Assessed by Serial Dilution. Means of Four Replicate Samples.

| *Fungi* | *No. of CFU $g^{-1}$ fresh weight tobacco* | |
|---|---|---|
| | *Burley* | *Virginia* |
| *Aspergillus* spp. | 110 | 200 |
| *A.amstelodami* | 25 | 80 |
| *A.niger* | 15 | 20 |
| *A.ruber* | < 10 | 10 |
| *Penicillium* spp. | 55 | 70 |
| *P.chrysogenum* | 10 | 23 |
| Others[a] | < 10 | < 10 |

[a] Includes *Chaetomium, Rhizopus, Trichoderma, Syncephalastrum, Scopulariopsis, Cladosporium* and *Mucor* spp.

0·85 $a_w$, 11–32 days at 0·80 $a_w$. *P.chrysogenum* isolates required 3–6 days at 0·85 $a_w$ and 19–29 days at 0·80 $a_w$.

Detailed studies of the dominant fungal species showed that growth of *A.amstelodami, A.ruber, A.fumigatus, A.niger, P.chrysogenum* and *P.viridicatum* was significantly influenced by water availability as well as nutrient composition. Growth rates were found to vary considerably at a set $a_w$ between malt, Virginia and Burley extract agars (Fig. 2). *A.ruber* and *A.amstelodami* grew best on malt extract agar over the range 0·75–0·995 $a_w$, followed by Burley and Virginia extracts (Fig. 2(a), (b)). Optimum growth of *A.amstelodami* was at 0·90 $a_w$ on malt extract but this changed to 0·95 $a_w$ on the tobacco extracts. Growth of *A.niger* was found to be almost 50% faster on Virginia than Burley extract agar at optimum $a_w$ (Fig. 2(c)). Furthermore, while 0·98 $a_w$ was optimum for growth of *A.niger* on malt and Burley extracts, it grew best at 0·995 $a_w$ on Virginia extract. With *A.fumigatus*, growth was again significantly slower on both Virginia and Burley extracts compared to that on malt extract agar (Fig. 2(d)). *P.chrysogenum* grew best on malt, followed by Virginia and Burley extract agars. As with *A.niger*, the $a_w$ for optimum growth changed from 0·98 on malt and Burley extract to 0·95 $a_w$ on Virginia extract (Fig. 2(e)). However, there was little difference in growth of *P.viridicatum* on the three nutrient substrates at the $a_w$ levels tested (Fig. 2(f)). Optimum $a_w$ for growth was 0·98 on all three media.

**TABLE 4**
Comparison of the Number of Isolates of Each Fungal Species Isolated from Burley and Virginia Tobaccos to Germinate or Grow at Different Water Activities ($a_w$).

| *Organism* | *Total number of isolates* | *No. of isolates tolerating each* $a_w$ | | | |
|---|---|---|---|---|---|
| | | *0·75* | *0·80* | *0·85* | *> 0·85* |
| *A.niger* | (16) | 0 | 4 | 9 | 3 |
| *A.amstelodami* | (14) | 5 | 8 | 1 | — |
| *A.versicolor* | ( 8) | 0 | 5 | 3 | — |
| *A.chevalieri* | ( 2) | 1 | 0 | 1 | — |
| *A.repens* | ( 1) | 1 | 0 | 0 | — |
| *A.flavus* | ( 2) | 0 | 1 | 1 | — |
| *A.fumigatus* | ( 2) | 0 | 0 | 2 | — |
| *A.ruber* | ( 4) | 0 | 4 | — | — |
| *A.awamori* | ( 1) | 0 | 0 | 1 | — |
| *A.sydowii* | ( 1) | 1 | — | — | — |
| *Aspergillus* spp. | (20) | 0 | 13 | 1 | 6 |
| *P.chrysogenum* | ( 6) | 0 | 2 | 4 | — |
| *P.viridicatum* | ( 2) | 0 | 0 | 2 | — |
| *P.crustosum* | ( 1) | 0 | 0 | 1 | — |
| *P.spinulosum* | ( 1) | 0 | 0 | 1 | — |
| *P.restrictum* | ( 2) | 0 | 2 | — | — |
| *Penicillium* spp. | ( 9) | 0 | 2 | 6 | 1 |
| *S.racemosum* | ( 1) | 0 | 0 | 0 | 1 |
| *S.brevicaulis* | ( 1) | 0 | 0 | 0 | 1 |
| *Trichoderma* sp. | ( 1) | 0 | 0 | 1 | 0 |
| *Rhizopus* sp. | ( 4) | 0 | 0 | 0 | 4 |
| *Chaetomium* sp. | ( 7) | 0 | 0 | 3 | 4 |

## Effect of tobacco type and water availability on moulding

Storage tests demonstrated that the mould-free shelf life of tobacco was increased by reducing the $a_w$, but that the $a_w$ level varied with tobacco type. Generally, the high-sugar tobaccos were more susceptible to mould damage than the low-sugar ones (Table 5). Oriental and Virginia tobaccos were both very susceptible, becoming visibly mouldy within eight days storage at 0·85–0·90 $a_w$, and within two and three weeks respectively at 0·80–0·85 $a_w$. Mould growth was inhibited for up to 3–4 months between 0·70 and 0·75 $a_w$, and by over a year at below 0·70 $a_w$.

An examination of the associated fungi at the time of visible moulding showed that the widest range of fungi were isolated from Indian and Virginia tobaccos (Table 6). Furthermore, a wider range of genera were

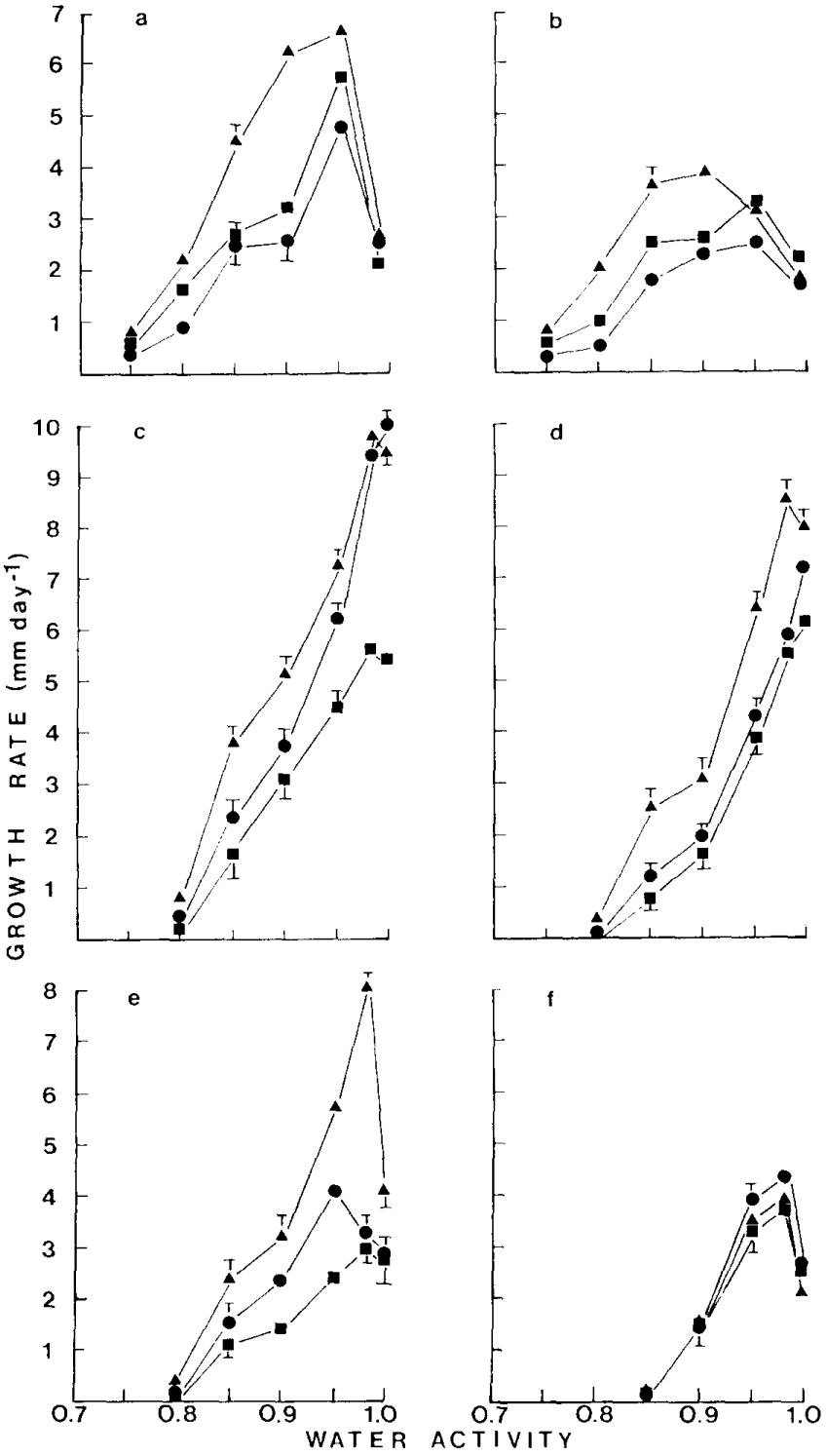


**Fig. 2.** The effect of water activity and substrate (malt extract (▲); Virginia extract (●); and Burley extract agars (■)) on growth of: (a) *Aspergillus ruber;* (b) *A.amstelodami;* (c) *A.niger;* (d) *A.fumigatus;* (e) *Penicillium chrysogenum;* (f) *P.viridicatum* at 30°C and pH 6·0. Bars indicate confidence limits ($P$ = 0·01). Bars are absent where variation was smaller than the size of symbol used.

**TABLE 5**
The Effect of Water Activity on the Mould-Free Storage Time (Days) of Five Different Tobaccos Stored at 30°C.

| *Tobacco type* / *Water activity* | *Mould-free storage time (days)* | | | | |
|---|---|---|---|---|---|
| | *Indian* | *Malawi* | *Oriental* | *Burley* | *Virginia* |
| 0·85–0·90 | 22 | 14 | 8 | 14 | 7 |
| 0·80–0·85 | 30 | 30 | 30 | 28 | 21 |
| 0·75–0·80 | 56 | 30 | 56 | 90 | 42 |
| 0·70–0·75 | 140 | 112 | 112 | 365 | 112 |
| 0·70 | 365 | 365 | 365 | 365 | 365 |

isolated from tobaccos which became mouldy above 0·85 $a_w$. Below 0·85 $a_w$ only *Aspergillus* species, especially members of the *A.glaucus* group, were recovered from all of the tobaccos.

## DISCUSSION

The moisture sorption isotherms obtained for different tobacco types in this study showed that isotherm position is correlated with total sugar content. Similar relationships have been shown previously for other tobacco types (Furusawa & Nozawa, 1966) and for food products (Wolf *et al.*, 1972). However, the moisture sorption isotherms of nominally the same tobacco type may differ as much as those between different tobacco types.

The differences found between the two high-sugar-content types, Oriental and Virginia, suggest that other factors beside sugar may play an important role in determining the moisture sorption isotherm. This difference is not due to a hysteresis effect as all types were conditioned by adsorption of water. It is possible however that these differences were due to the physical characteristics of the leaf. For example, it has been previously demonstrated that surface structural effects such as capillarity, type and distribution of hydrophilic groups can influence water sorption isotherms in such systems (Labuza & Ratman, 1968; Bluestein & Labuza, 1972, 1975) and that physical characteristics can influence moisture holding capacity (Bacot, 1960).

These results suggest that recommendations of moisture content for safe storage of tobacco need careful consideration, and must be linked to the actual $a_w$ of the tobacco type at each moisture content level. At present, the recommended safe moisture content limit of 15% corresponds

**TABLE 6**
The Dominant Fungal Genera and Species Isolated From Different Tobaccos Stored at Different Water Activities for up to One Year at 30°C.

| | *Tobacco type* | | | | |
|---|---|---|---|---|---|
| *Water activity* | *Indian* | *Malawi* | *Oriental* | *Virginia* | *Burley* |
| 0·85–0·90 | *Monascus ruber*<br>*A.glaucus* spp.<br>*A.fumigatus*<br>*A.flavus*<br>*Aspergillus* spp. | *Aspergillus* spp. | *Chaetomium globosum*<br>*A.glaucus* spp. | *A.glaucus* spp.<br>*A.niger*<br>*Penicillium* spp. | *Aspergillus* spp.<br>*A.glaucus* spp. |
| 0·80–0·85 | *A.glaucus* spp. | *A.glaucus* spp. | *Aspergillus* spp. | *A.glaucus* spp. | *A.glaucus* spp. |
| 0·70–0·80 | *A.glaucus* spp. | *A.glaucus* spp. | *A.glaucus* spp. | N.G. | N.G. |

N.G., No visible growth during storage test.

to between 0·69 and 0·79 $a_w$ for the tobaccos tested. This range would certainly allow fungal spoilage to be initiated, particularly at ambient storage temperatures of 20–30°C.

*Aspergillus* and *Penicillium* spp. were most frequently isolated from Virginia and Burley tobaccos. Although the numbers of fungi isolated from Virginia were higher than those from Burley, the relative proportions of each group of species were similar for the two tobacco types. The range of species recovered was similar to that previously isolated by other workers (Welty & Lucas, 1968; Bell, 1971; Welty & Vickroy, 1975). However, the dominant fungi can vary considerably due to the handling and storage methods used (Bell, 1971).

Of about 100 isolates examined, almost 80% were able to germinate or grow at or below 0·85 $a_w$ and could be considered to be xerophiles, as defined by Pitt (1975). The majority of species and isolates studied were therefore potential deteriogens of tobacco stored below 0·85 $a_w$, which is equivalent to 31% in Virginia and 24% moisture content in Burley. In this study 0·75, 0·80 and 0·85 were therefore chosen as critical water availabilities in the context of tobacco storage and spoilage. While there was some difference in the ability of strains of the same species to tolerate one of these $a_w$ levels, only *Aspergillus glaucus* group species were able to germinate and grow at 0·75 $a_w$. Other *Aspergillus* and *Penicillium* spp. grew at 0·80 $a_w$. These two genera would therefore include almost all the fungi which might be expected to cause spoilage at these levels of $a_w$. This was to some extent confirmed by the storage tests where between 0·70 and 0·80 $a_w$ *A.glaucus* group spp. only were isolated. Between 0·80 and 0·85 $a_w$ *A.glaucus* group spp. and other *Aspergillus* spp. occurred, while between 0·85 and 0·90 $a_w$ *Penicillium* and other non-xerophilic fungi were dominant. Previously, *A.glaucus* group were the only species isolated from Virginia, stored at 26·8%, and Burley, stored at 19·4% moisture content (Welty & Weeks, 1973), i.e. equivalent to 0·75 $a_w$. Further, *Penicillium* spp. were rarely isolated from flue-cured tobacco stored at 24% moisture content (= 0·79 $a_w$) (Welty & Lucas, 1968). More recently, McKee *et al.* (1984) found that moisture contents below the range 20·4–24·5% were critical for the safe storage of different crops and grades of tobacco. However, the equivalent $a_w$ levels were not determined for the different crop types.

The storage experiments also demonstrated the direct relationship between $a_w$ and length of time tobacco could be stored safely. Between 0·75 and 0·85 $a_w$, fungal spoilage would be initiated rapidly with visible moulding within 1–2 months. Interaction between $a_w$ and tobacco type can further influence the time for safe storage.

The results obtained *in vitro* for germination and growth at 0·75, 0·80

and 0·85 $a_w$ do not represent the absolute limits of growth for the fungal species studied. Previously, isolates of *A.chevalieri, A.amstelodami* and *A.repens* have been reported to be able to grow below 0·75 $a_w$ (Pitt, 1975) while *A.versicolor* has been found to have a minimum $a_w$ for germination of 0·78 (Magan & Lacey, 1984*a*).

With the exception of *P.viridicatum,* growth of the *Aspergillus* and *Penicillium* spp. tested was significantly slower on the tobacco extract than on the richer malt extract agar. There were also some differences between fungi in their response to the two types of tobacco agars, Virginia and Burley. For *A.niger, A.fumigatus,* and *P.chrysogenum,* growth was faster on Virginia extract than Burley extract agar. Only *A.ruber* and *P.viridicatum* had the same optimum $a_w$ for growth on all three substrates. For *A.niger* and *P.chrysogenum* the $a_w$ optimum for growth changed on Virginia extract agar. Nutrient substrate has previously been shown to have an influence on the growth rate and the $a_w$ range for germination and growth of spoilage fungi (Snow, 1949; Troller & Christian, 1978) as well as interactions between fungi (Magan & Lacey, 1984*b*). These results indicate that there is therefore an interaction between $a_w$ and substrate composition, and more relevant information on the ability of tobacco spoilage fungi to grow at low $a_w$ may be obtained by using tobacco extract agars rather than richer media. The more rapid growth of fungi on the Virginia extract suggests that spread and penetration of bales of flue-cured tobacco of this type may be more rapid than occurs in Burley bales. This is to some extent borne out by the more rapid spoilage of Virginia tobacco when compared to Burley in the storage experiments.

This work demonstrates that combining information on water sorption isotherms with that on the $a_w$ limits of the most important spoilage fungi can enable more accurate prediction of safe storage limits for different types of flue-cured tobaccos. However, it is important to bear in mind the hysteresis effect when using water sorption isotherms in both flue-cured and manufactured tobacco as water is often added or removed at various stages during the post-harvest manipulation of tobacco. Furthermore, different tobaccos have distinct characteristics and may interact with spoilage fungi in different ways making it necessary to consider each type separately. There is clearly a complex interaction between tobacco type, $a_w$, other environmental conditions as well as added preservatives and casing solutions which will influence the activity of spoilage fungi.

## ACKNOWLEDGEMENT

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