THE EFFECT OF WATER ACTIVITY AND TEMPERATURE ON MYCOFLORA AND DRY MATTER LOSS OF RAPESEED

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ABSTRACT

Laboratory studies were carried out to determine effect of environmental factors on dry matter loss, fungal colonisation and germination of rapeseed over periods of up to 28 days.

The respiration of rapeseed stored for up to 6-7 days varied significantly with both temperature (20-30°C) and water activity (a_w). Longer periods of storage showed that at high a_w (0.95 and 0.98) the percentage dry matter loss varied from about 2% at 15°C to 5.5% at 30oC after 28 days. However, at lower a_w levels (0.85 and 0.90) this was reduced to between 0.5 and 1.25%. Direct plating of rapeseed onto media of the same a_ws showed that both temperature, a_w and length of storage affected the dominant fungi colonising the rapeseed over this storage period. For example, at 15°C Penicillium and Mucor spp. were dominant at 0.98 a_w; Penicillium spp. at 0.95 a_w; and Penicillium spp., Eurotium spp. and Wallemia sebi at 0.90 and 0.85 a_w. These patterns were altered at 25 and 30°C.

Germination of seeds was significantly reduced during storage at all temperatures and 0.95-0.98 a_w. At 0.85 a_w, moulding had less effect on seed germination.

INTRODUCTION

The development of fungi in stored agricultural crops is closely related to the water content (water availability) at harvest, the storage temperature and the intergranular atmosphere (Sinha, 1973; Magan and Lacey, 1989). The contaminant fungi have distinctive ranges of temperature and water availability (a_w) for growth (Magan and Lacey, 1984a) and this will influence both the rate of spoilage and the extent of dry matter losses which might occur. Based on mycological and biochemical examination of farm-stored rapeseed Mills and Sinha (1980) suggested conditions under which rapeseed could be safely stored in Canada while a study in the U.K. determined the time periods available for drying prior to the appearence of visible moulding (Burrell et al, 1980). However, the criterion of visible moulding gives very little indication of the level of grain deterioration and loss in quality. For example, Seitz et al (1982) by measuring respiration of maize showed that fungal invasion and aflatoxin content could reach unacceptable levels before the grain had lost 0.5% dry matter and mould growth became visible. No detailed information is available on the effect of environmental factors on respiration, dry matter losses and moulding of rapeseed.

The objectives of this study were to determine the effect of water availability and temperature on dry matter losses, patterns of mould development and germination of rapeseed during short periods of storage.

MATERIALS AND METHODS

Rapeseed water contents

To manipulate the water availability of rapeseed accurately, moisture sorption curves were initially determined. Known amounts of distilled water were added to 10-20 g subsamples of cv. Libravo and allowed to equilibrate in a 5°C fridge, with regular mixing, over a 24h period. The water content (wet weight basis) of 10 g subsamples was determined in a drying oven at 105°C for 17 hrs. The a_w of 5 g subsamples was determined with a Humidat IC II (Novasina AG, Switzerland). Adsorption curves were determined at 15, 25 and 30°C. These curves were subsequently used to accurately modify the a_w of rapeseed samples for detailed experiments.

Respiration studies

Rapeseed (cv Libravo) was modified to 0.95, 0.90, 0.85 and 0.70 a_w by the addition of sterile distilled water using the isotherms. These a_ws approximate to about 25-26%, 15-16%, 12-13.5% and 7.5-8.5% water content, respectively. Fifteen gram samples were weighed out and placed in a respirometer unit (Tribe and Maynard, 1989). The details of the automatic respirometer system and its use are described by Hamer, Lacey and Magan, this volume). The respiration of treatments and replicates were monitored at hourly intervals for up to 6-7 days. The amounts of oxygen utilized kg^{-1} rapeseed were determind.

Dry matter loss

Rapeseed was equilibrated to 0.98, 0.95, 0.90 and 0.85 a_w by addition of distilled water and reference to the moisture sorption curves at each temperature. A total of twenty five relicate subsamples of 20g at each a_w and temperature were carefully weighed into small clean beakers and placed in large sandwich boxes. To maintain the equilibrium relative humidity 200 ml approriate salt solutions were placed in the experimental boxes. The boxes were enclosed in polyethylene bags which were loosely tied to enable gas exchange. The treatments were incubated at 15, 20, 25 and 30 C. Every seven days, three replicate beakers were removed from each treatment and dried at 105°C to determine dry matter and another three replicates for assessment of fungal colonisation and seed germination. The experiments were repeated twice.

Fungal colonisation

Malt extract agar (2%) was modified to the same a_w as the treatments with glycerol (Dallyn and Fox, 1980) and prepard in 9cm Petri dishes. For each replicate a total of 50 seeds were plated out, 10 per plate. After incubation for 7-21 days at the same experimental temperatures the fungal genera and species were identified and enumerated.

Germination

Germination of 100 seeeds per replicate was determined on moist filter paper at 20-25°C and assessed after approximately 8-10 days.

RESULTS AND DISCUSSION

Respiration of the rapeseed was influenced by both a_w and temperature. At 25 and 30°C and 0.95 a_w respiration increased linearly with time. The amount of oygen utilized decreased significantly at 0.90 and 0.85 a_w, with very low respiration rates at 0.70 a_w. The rate of oxygen utilization by a kilogram of rapeseed h^{-1} was subsequently determine from results obtained at 20, 25 and 30°C (Table 1). This summary table shows that regardless of temperature the rate of respiration of rapeseed at 0.70, at which practically no mould growth occurs, was very low. However, this changes rapidly at 0.85 a_w where differences in respiration rate between temperatures becomes evident. This marked increase in oxygen utilization must in part be due to fungal activity, although no visible growth could be seen in the treatments except for clumping of seeds at 0.95 a_w at the end of the experiment. Experiments with sterile rapeseed and individual spoilage fungi suggest that at 0.90 and 0.95 a_w fungal respiration may be an important part of the total respiration (Magan, unpublished data) although Seitz et al. (1982b) suggested that the microflora of moist maize contributed less to respiration than the maize grain itself. Earlier experiments with sterile maize seed suggestd that fungi contributed about 10% of the total respiration (Woostock and Combs, 1965).

Over 28 day storage periods, the percentage dry matter of rapeseed lost varied considerably at different temperatures and a_ws (Table 2). The optimum dry matter loss occurred at 0.98 a_w at all temperatures. Visible moulding was observed within 7-14 days at this aw in all treatment temperatures. After 28 days, over 5.0% dry matter was lost at 30°C and over 4.0% at 20 and 25°C. At 0.98, 0.95 and 0.90 a_w and 20,25 and 30°C dry matter loss was rapid with at least 0.1% being lost after 7 days storage. In general, the first indication of moulding was the clumping of seeds, followed by the presence of mycelium amd sporophores of fungi when viewed with a dissecting microscope. Visible moulding was delayed most in the 0.85 a_w treatments as found previously by Burrell et al (1980). However, in their studies no information was obtained on the rate of dry matter loss under different environmental conditions.

The aw and temperature of storage has a marked effect on the colonization of the rapeseed by spoilage fungi. The results obtained at 15 and 25°C are shown in Fig.1 and Fig.2. At 15°C, Penicillium spp., Mucor and initially Cladosporium spp. were predominantly isolated during the 28 day storage period. However, at 0.95 a_w, Penicillium spp. were the only genus isolated consistently. However, at 0.90 a_w, Eurotium spp. (Aspergillus glaucus group) and Wallemia sebi were also isolated. The percentage rapeseed colonised by Penicillium spp. was lower than at 0.98 and 0.95 a_w. At 0.85 a_w, the lowest water availability tested, colonisation with Wallemia sebi increased with time while that of Penicillium spp. decreased from 60% to about 20% after 14 days storage. At 25°C a similar range of fungi were found at 0.98 a_w with Penicillium spp., Mucor and Alternaria spp. being most commonly isolated. At 0.95 and 0.90 a_w, Penicillium and Eurotium spp. were dominant with low levels of Alternaria and Aspergillus candidus being present. At 0.85 a_w, colonisation with Eurotium spp. increased from less than 10% initially to 100% after 21 days storage. The percentage rapeseed carrying Penicillium spp. was reduced from 65 to 40% while W.sebi was still present on some seed after 21 days storage.

Figure 1. The changes in patterns of colonisation of rapeseed during storage at different water activities and 15°C. Key to fungi: Alt, Alternaria alternata; A.can, Aspergillus candidus; A.ver, Aspergillus versicolor; Clado, Cladosporium spp.; Eur, Eurotium spp.; Muc, Mucor; W.sebi, Wallemia sebi.

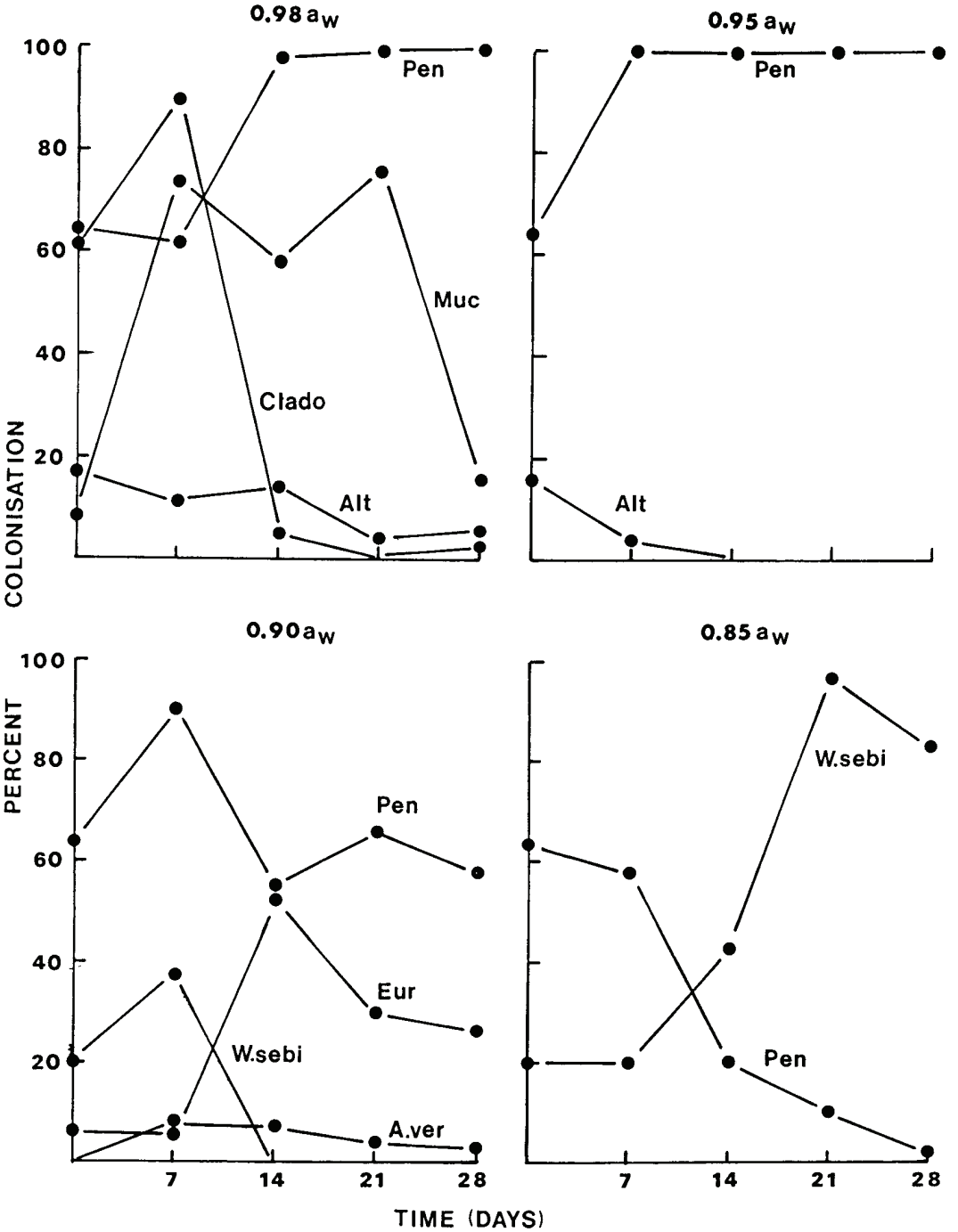

Figure 2. Changes in patterns of colonisation of rapeseed during storage at different water activities and 25°C. For key to fungi see Fig. 1.

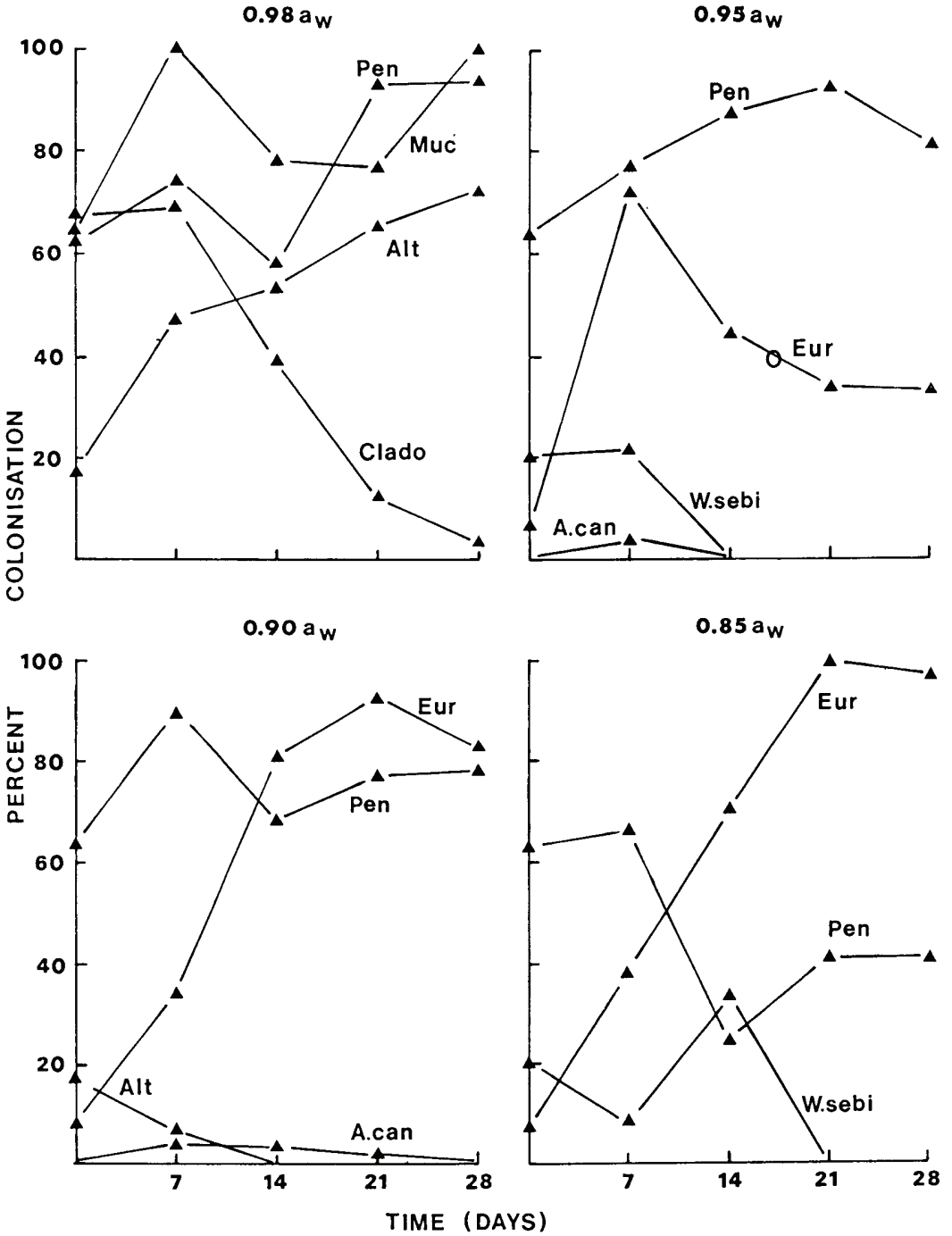

Table I. The amounts of oxygen utilized kg h^{-1} by rapeseed at four different water availabilities and three temperatures.

Water activity (a_w)	Temperature (°C) 20	25	30
0.95	57.48 + 3.20	70.58 + 1.92	70.40 + 3.18
0.90	21.26 + 1.37	32.23 + 1.49	49.07 + 1.36
0.85	5.68 + 0.72	9.98 + 1.04	13.43 + 0.77
0.70	1.75 + 0.07	2.18 + 0.53	1.50 + 0.08

Table II. The effect of steady state water activity and temperature conditions on the percentage (%) dry matter of rapeseed over 28 days. The analyses were carried out on actual dry matter loss and the mean % determined.

Temperature (°C) / Time (days) / Water activity (a_w)	7	14	21	28	L.S.D. (P = 0.05)
15					
0.98	0.55	0.80	1.00	2.35	0.54
0.95	< 0.10	0.84	1.25	1.48	0.26
0.90	0	0.40	0.65	0.73	0.21
0.85	0	< 0.10	0.45	0.45	0.14
20					
0.98	1.91	2.40	2.73	4.42	0.72
0.95	0.50	0.74	1.02	1.10	0.30
0.90	0.20	0.65	1.15	1.23	0.17
0.85	0	< 0.10	0.30	0.75	0.12
25					
0.98	1.85	2.30	3.10	4.91	0.50
0.95	0.55	1.10	2.55	3.20	1.00
0.90	0.22	0.30	0.50	0.85	0.14
0.85	0	0.15	0.45	0.90	0.12
30					
0.98	2.75	3.30	3.60	5.45	0.72
0.95	0.39	1.30	2.33	2.80	0.48
0.90	0.40	0.42	0.55	1.25	0.26
0.85	< 0.10	0.11	0.40	0.60	0.15

Under steady state conditions of a_w and temperature the patterns of fungal colonisation and succession were quite complex. Interactions between species of spoilage fungi varies with environmental conditions and substrate (Magan and Lacey, 1984b) and will probably influence the dry matter losses observed. Different species are responsible for spoilage at different a_w and temperatures. While Penicillium spp. may be particularly

important at low temperatures, Eurotium spp., A.candidus, A.flavus, A.versicolor and Mucor spp. may be more important at above 25°C. The range of fungi isolated in this study differs slightly from that obtained in Canada, where Cephalosporium spp. were present on up to 60% of rapeseed during storage at between 0.70 and 0.90 a_w over the same storage period (Mills and Sinha, 1980). The changes in fungal colonisation of rapeseed and differences in dry matter losses suggest that fungi may differ considerably in their ability to produce the necessary enzymes, particularly lipases, involved in spoilage of rapeseed and other oilseeds.

The effect of treatments on germination of rapeseed is shown in Table 3. Although the germination of the freshly harvested rapeseed was about 75% this was affected by some treatments. At 15°C, these was little change in the seed germination 28 day period except at the highest a_w tested, 0.95, where germination decreased markedly to about 20-25% after 21-28 days storage. At 25 and 30°C a marked decrease in germination occurred at 0.95 and 0.90 a_w. Since different fungi dominate the stored rapeseed under different environmental conditions, some species may more effectively infect the embryo than others. The fact that germination was particulary affected at 0.95 and 0.90 a_w suggests that Aspergillus and Penicillium spp. may be particularly effective in producing the enzymes to infect the seed and embryo. These results differ from that of Burrell et al (1980) who found little effect on germination of rapeseed after four weeks storage, with only some reduction of germination at high water availabilities after 5 months storage.

Acknowledgements

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Table III. The effect of temperature and water availability on percentage (%) germination of rapeseed stored for up to 28 days.

Temperature (°C)			15		
Time (in days)	0	7	14	21	28
Water activity (aw)					
0.98	75	59	64	26	21
0.95		55	62	65	56
0.90		63	68	64	61
0.85		65	68	64	61
			20		
0.98	75	61	67	60	43
0.95		75	84	26	10
0.90		56	62	46	38
0.85		68	76	82	52
			30		
0.98	75	71	64	58	48
0.95		56	20	30	8
0.90		56	26	50	16
0.85		64	68	76	80

REFERENCES

Burrell, N.J., Knight, G.P., Armitage, D.M. and Hill, S.T. (1980). Determination of the time available for drying rapeseed before the appearence of surface moulds. J. stored. Prod. Res. 16, 115-118.

Dallyn, H. and Fox, A. (1980). Spoilage of materials of reduced water activity by xerophilic fungi. Soc. Appl. Bact. Tech. Series 15, 129-139.

Magan, N. and Lacey, J. (1984a). The effect of temperature and pH on the water relations of field and storage fungi. Trans. Br. Mycol. Soc. 82, 71-81.

Magan, N. and Lacey, J. (1984b). The effect of water activity, temperature and substrate on interactions between field and storage fungi. Trans. Br. Mycol. Soc. 82, 83-93.

Magan, N. and Lacey, J. (1988). Ecological determinants of mould growth in stored grain. Int. J. Fd. Mirobiol. 7, 245-256.

Mills, J.T. and Sinha, R.N. (1980). Safe storage periods for farm-stored rapeseed based on mycological and biochemical assessment. Phytopathology 70, 541-547.

Seitz, L.M., Sauer, D.B., Mohr, H.E. and Aldis, D.F. (1982a). Fungal growth and dry matter loss during bin storage of high moisture corn. Cereal Chem. 59, 9-14.

Seitz, L.M., Sauer, D.B. and Mohr, H.E. (1982b). Storage of high moisture corn: fungal growth and dry matter loss. Cereal Chem. 59, 100-105.

Sinha, R.N. (1973). Grain storage: part of a system. (Ed. R.N.Sinha and W.E.Muir), pp. 15-48. Westport, Connecticut, Avi Publishing Co., U.S.A.

Tribe, H.T. and Maynard, P. (1988). A new automatic respirometer. Proc. of Royal Soc. Edin. 94, 178-180.

Woodstock, L.W. and Combs, M.F. (1965). Effects of gamma-irradiation of corn seed on the respiration and growth of the seedling. Amer. J. Bot. 52, 563-569.

LES EFFETS DE L'ACTIVITE DE L'EAU ET DE LA TEMPERATURE SUR LA MYCOFLORE ET LA PERTE EN MATIERE SECHE DE LA GRAINE DE COLZA

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RESUME

Des études de laboratoire ont été entreprises pour déterminer l'effet des facteurs environnementaux sur les pertes de matière sèche, la colonisation fongique et la germination des graines de colza sur une période de 28 jours.

Pour une activité de l'eau élevée (0,95 à 0,98 a_w), le pourcentage de perte de matière sèche a varié d'à peu près 2 % à 15 C jusqu'à 5,5 % à 30 C après 28 jours de stockage. Cependant, à 0,85 et 0,90 a_w, il a été réduit entre 0,5 et 1,25 %. L'application de la méthode de révélation directe sur boîte, sur des milieux à même a_w montre qu'à la fois la température, l'a_w et la durée de stockage ont affecté les champignons colonisateurs sur cette période de 28 jours. Par exemple, à 15 C, *Penicillium* et *Mucor spp.* étaient dominants à 0,98 a_w, *Penicillium* spp. à 0,95 a_w et *Penicillium* spp., *Eurotium* spp. et *Wallemia sebi* à 0,90 et 0,85 a_w. Ces tendances étaient légèrement moins nettes à 25 et 30 C. La germination a diminué significativement au cours du stockage à toutes les températures et à 0,95- 0,98 a_w. A 0,85 a_w, les moisissures avaient moins d'effets sur la germination des graines.

L'évaluation du rôle de chaque espèce de champignon dans l'envahissement mycélien et dans les pertes de matière sèche pour les graines oléagineuses est en cours d'évaluation.