

Occurrence of fungal species in stored alfalfa forage as influenced by moisture content at baling and temperature during storage

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Undi, M., Wittenberg, K. M. and Holliday, N. J. 1997. **Occurrence of fungal species in stored alfalfa forage as influenced by moisture content at baling and temperature during storage.** *Can. J. Anim. Sci.* **77**: 95–103. A study was conducted to evaluate the effect of moisture content at baling on fungal growth during storage of alfalfa forage. Alfalfa forage was baled at DM contents of 64.1–66.2% (Low), 71.9–73.2% (Medium) and 75.4–77.4% (High) and was sampled 1, 2, 3, 4, 5, 6, 7, 8, 9, 14, 21, 35 and 60 d after storage. For each sample, abundance of fungal and yeast species was assessed by plating on media. Total fungal counts, number of species, species diversity, and species dominance were subjected to analysis of variance, and variation in the fungal assemblages was characterized by ordination. Total fungal counts, number of species, species diversity, and species dominance were not influenced ($P > 0.05$) by moisture content in the early (days 1 to 8) storage phase. Moisture content at baling did influence ($P < 0.05$) total counts, number of species, and species dominance in the latter phase (days 9 to 60) of storage. Total fungal counts were highest in Low DM forage, and number of species highest in Medium DM forage. Species dominance was highest in High DM forage. Low DM forage was associated with *Aspergillus fumigatus*, *Mucor* spp., *Absidia* spp., *Emericella nidulans*, and thermotolerant hyphomycetes. *Aspergillus repens*, *Absidia* spp. and some yeasts were more predominant in Medium and High DM forages. Moisture content and temperature were related to species assemblages during storage but water-soluble carbohydrate concentration, crude protein concentration and total bacteria counts were not. "Field" fungi, *Phoma*, *Alternaria*, *Cladosporium* spp. and most yeasts were eliminated within 8 d of storage. Physical conditions created in the early stages of storage likely affected fungal growth in the later storage phase.

Key words: Moisture content, temperature, storage, alfalfa hay, fungal species, diversity

Undi, M., Wittenberg, K. M. et Holliday, N. J. 1997. **Fréquence d'apparition de diverses espèces fongiques dans le foin de luzerne selon sa teneur en eau au moment de sa mise en balles et de la température de conservation.** *Can. J. Anim. Sci.* **77**: 95–103. Nous avons observé l'effet de la teneur en eau du foin de luzerne à la mise en balles sur la croissance fongique durant la phase de conservation du foin. Les teneurs en m.s. lors de la mise en balles étaient, respectivement, de 64,1–66,2 % (basse), 71,9–73,2 % (moyenne) et 75,4–77,4 % (haute). Des prélèvements de foin étaient effectués après 1, 2, 3, 4, 5, 6, 7, 8, 9, 14, 21, 35 et 60 j de stockage. L'abondance des champignons et des levures était évaluée par mise en culture sur boîtes de Pétri. Les numérations fongiques totales, le nombre d'espèces observées et leur diversité ainsi que l'indice de dominance étaient soumis à l'analyse de la variance et les variations du complexe fongique étaient caractérisées par ordination. Aucun de ces quatre caractères n'était pas influencé ($P > 0,05$) par la teneur en eau du fourrage dans la phase initiale de conservation (j 1 à j 8). La teneur en eau, par ailleurs, exerçait un effet significatif ($P < 0,05$) sur les numérations totales, sur le nombre d'espèces et sur l'indice de dominance dans le reste de la période (j 9 à j 60). Les numérations fongiques totales les plus fortes se retrouvaient dans le foin à basse teneur en m.s. (BMS) et le nombre le plus élevé d'espèces présentes dans le foin à teneur moyenne en m.s. (MMS). L'indice de dominance était particulièrement élevé dans le foin à haute teneur en m.s. (HMS). Le foin BMS était colonisé par les espèces *Aspergillus fumigatus*, *Mucor* spp., *Absidia* spp., *Emericella nidulans* et par les hyphomycètes thermotolérants, alors que *Aspergillus repens*, *Absidia* spp., et certaines levures étaient plus abondants dans le foin à teneurs moyenne et élevée en m.s. On observait un rapport entre la composition de la microflore fongique durant la période de conservation et la teneur en eau lors de la mise en balles et la température interne du foin, mais il n'y avait pas de rapport avec les concentrations de glucides hydrosolubles et de protéine brute ou avec les numérations bactériennes totales. Les champignons d'origine : *Phoma*, *Alternaria*, *Cladosporium* sps. étaient éliminés dans les huit premiers jours de stockage. Les conditions physiques créées dans les débuts du stockage influençaient vraisemblablement sur la croissance fongique dans la phase ultérieure.

Mots clés: Teneur en eau, température, stockage, foin de luzerne, espèce fongique, diversité

Approximately 5.8 million ha were used to produce about 29 million t of tame hay in Canada in 1991 (Statistics Canada 1992). Fifty-six percent of the land was used to produce about 16.4 million t of alfalfa and alfalfa-grass hay, with an estimated value of approximately \$1 billion. Only a small fraction (0.2%) of total hay produced was exported, the rest presumably being used to feed livestock on the farm

or sold within the country. One major determinant of hay price, for export or for local trade, is hay quality, and quality evaluation takes into account nutrient composition as well as visual assessment for color and mold.

Moldy hay poses a risk both to animals that are fed, and to individuals who handle hay. Mycotic abortion and mycotic mastitis in cattle, and farmer's lung in humans have

been associated with feeding and handling of moldy hay (Lacey 1975; Knudtson and Kirkbride 1992). Storage fungi in hay are potential mycotoxin producers, which is a further source of concern. Thus, there is a need to characterize fungal growth during storage of hay.

The information available on fungal growth during hay storage has been confined to grass forages (Gregory et al. 1963; Breton and Zwaenepoel 1991). Such work has shown that, before harvest and during field wilting, field fungal species such as *Clasosporium*, *Alternaria*, *Fusarium* and *Phoma* predominate in grass forage although some storage species, namely, *Penicillium*, *Mucor*, *A. glaucus*, and *A. flavus* also have been isolated during wilting (Pizarro and Warboys 1980; Clevstrom and Ljunggren 1984).

Field fungi can be succeeded by storage fungi during storage, and environmental factors such as moisture content and temperature may ultimately determine the fungal species that predominate in stored hay. Common storage fungi include species of *Aspergillus*, *Mucor* and *Absidia*. The *Aspergillus glaucus* group, mainly *A. repens* and *A. amstelodami*, predominated in grass forage stored at 75% DM, whereas storage at lower DM contents resulted in dominance of thermotolerant *A. fumigatus*, *A. versicolor*, *Humicola lanuginosa*, and *Mucor* spp. (Gregory et al. 1963; Lacey 1975; Kaspersson et al. 1984; Breton and Zwaenepoel 1991).

In North America, where alfalfa is used extensively in the production of high-quality hay, little effort has been directed toward characterization of fungal growth during storage of this forage crop. The purpose of this work was to evaluate the effect of moisture content at baling on population ecology of fungal species in stored alfalfa forage.

MATERIALS AND METHODS

First cut alfalfa forage (cv. Arrow) from a 15-ha weed-free stand was used for this study. The stand was cut at 30% bloom into 34 swaths using a John Deere mower-conditioner (2.74-m swath width). Swaths were randomly assigned to three treatments. To reduce border effects, swaths on the edge of the field were not used. The entire field was cut on the same day, 22 July 1991. Forage was left to wilt in the field for at least 48 h before baling. During wilting, DM was monitored using a microwave oven. Drying conditions were good, with maximum temperatures averaging 25.5°C for the 4 d during forage wilting, approximately 14 h of sunshine per day, and no precipitation (Climatological data, Glenlea Research Station; Environment Canada).

Forage was baled (John Deere 336 baler) into small square bales at DM contents of 64.1–66.2, 71.9–73.2 and 75.4–77.4% and designated as Low, Medium and High DM forage, respectively. Low DM forage was baled on 24 July starting at 13:00 h. Medium and High DM forages were baled the following day starting at 12:00 h and 15:15 h, respectively. High DM forage was baled at a lower DM content than was originally intended due to a forecast for rain in the evening of the same day. Two 74-bale stacks of each forage treatment were made using a bale wagon (John Deere 1037) with the fourth layer of each stack tied. Stacks were stored in a pole shed structure and were placed immediately adjacent to each other with no space between them.

Sample Collection

Four bales were removed from inside the stack for measurement of temperature. Thermocouple wires were inserted into these bales after which the bales were returned to their original position within the stack, which was a minimum of one bale width from the stack exterior. The bale temperatures were read daily for 35 d, and then on days 45, 50 and 60. A Trendicator (Model 400A; Doric Scientific Div., Emerson Electric Co. San Diego, CA), calibrated before use by reading off thermocouple wires maintained in water at known temperatures, was used. All thermocouple wires were confirmed to be functioning normally prior to the study.

Two other bales were selected at random from each DM level-stack combination for successive sampling during storage. These bales were positioned one bale width from the stack exterior. The bales were core-sampled (one core per bale) using a Penn State core-sampler at the time of stacking and on days 2, 3, 4, 5, 6, 7, 8, 9, 14, 21, 35 and 60. Day 1 was considered to be the day of stacking. To prevent cross-contamination between samples, the core pipe and tip were disinfected with a 70:30 ethanol:water solution between core samplings. Samples were transferred aseptically to Whirlpac® bags and placed in a container on ice immediately after collection for transport to the laboratory.

Day 1 core sampling was done approximately 3.5 h after baling was initiated with the Low DM forage treatment. Medium and High DM hay bales were core sampled 4 h and 2.75 h after baling was initiated, respectively. Samples were taken at noon for all forage treatments from day 3 onward. One portion of each bale sample was used for microbiological analysis. The remainder was frozen (–20°C) for subsequent determination of DM by freeze drying, crude protein by Micro-Kjeldahl, and soluble carbohydrates as previously described by Wittenberg (1994). Forage pH was determined by placing 12.5 g forage into 50 mL distilled water for 2 h and determining pH of the decanted liquid.

Sample Preparation

Samples were stored at 4°C until time of processing, which was completed within 24 h of sample collection. A known amount (2 g) of sample was removed from Whirlpac® bags using sterile forceps and placed into sterile 50-mL Kimax test tubes with caps. Fifteen millilitres of sterile aqueous wash solution (5 g peptone, 3 g yeast extract and 20 mL glycerol L⁻¹) were dispensed into each tube. Tubes were sealed, placed onto a rack and suspended horizontally in a controlled-environment incubator-shaker (New Brunswick Scientific Co., Edison, NJ) and shaken at 400 rpm for 5 min at room temperature. The rack was then flipped and the process repeated. The wash solution was decanted and 30 mL of sterile water was added to each tube. This suspension was centrifuged at 21 × g for 10 min, after which the water was decanted. This process was repeated three times, then another 15 mL of wash solution was added and the suspensions were centrifuged at 21 × g for 5 min. All but 1 mL of supernatant was decanted from each tube. Tubes were then centrifuged at 8900 × g for 20 min to remove the remaining wash solution, leaving a pellet. Approximately 0.15 mL of

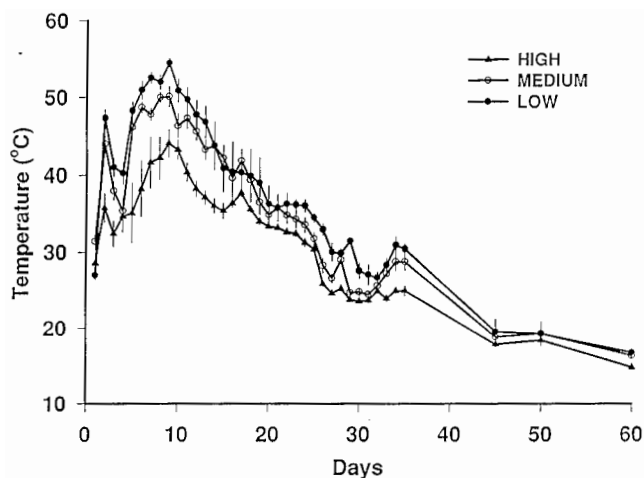


Fig. 1. Temperature changes during the storage of alfalfa forage baled at Low (64.1–66.2%), Medium (71.9–73.2%) and High (75.4–77.4%) DM contents.

Repeated measures analysis of variance was used to determine the effects of moisture content at baling on total fungal counts, number of species, the log series α index and the Berger-Parker index. Total counts were transformed to $\log(e)$ before analysis. Analysis was carried out using SYSTAT (Wilkinson 1988), except for the Berger-Parker index, which was analysed using SAS Institute, Inc. (1985) software.

The storage period was considered to consist of two phases, an early phase, which was from the day of baling (day 1) to day 8. The later storage phase was from day 9 to the last sampling day (day 60). This division was based on initial analysis of the communities for the entire storage period and on temperature during storage. Days 1 to 8 of storage were marked by increasing temperatures until peak temperatures were reached on about day 9 (Fig. 1). These storage phases were also used when the data for sites and fungal species were subjected to correspondence analysis (CA) and canonical correspondence analysis (CCA) using CANOCO software (ter Braak 1987–1992). Environmental variables tested in CCA included moisture content, crude protein, total bacteria, water-soluble carbohydrate, and temperature.

RESULTS

Heating in forage treatments, as assessed by temperature, began immediately following stacking and reached a transient peak after 2 d in storage (Fig. 1). At this peak, temperature of Low DM forage was higher ($P < 0.05$) than that of Medium DM forage and temperature of Medium DM forage was higher ($P < 0.05$) than that of High DM forage. Second peak temperatures occurred around day 9 (Fig. 1) and again were higher ($P < 0.05$) for Low DM forage than for Medium or High DM forages. Moisture loss was most rapid in Low DM forage, such that by day 14 of storage, moisture content had declined significantly in this treatment. Despite the more rapid rate of moisture loss, day 60 moisture content for Low DM was still higher than that of the other treatments (Fig. 2).

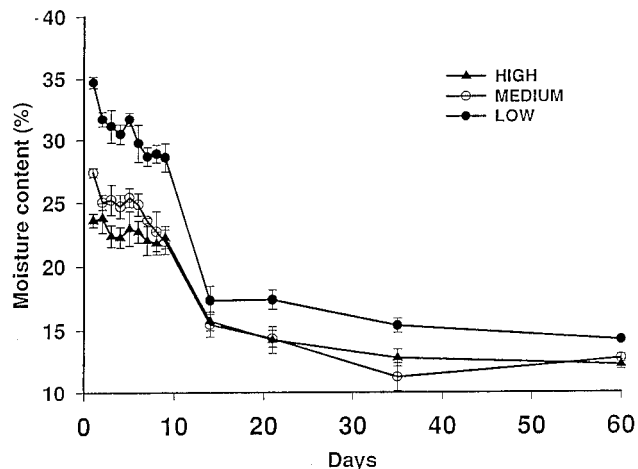


Fig. 2. Moisture change during the storage of alfalfa forage baled and stored at Low (64.1–66.2%), Medium (71.9–73.2%) and High (75.4–77.4%) DM contents.

Total bacterial counts averaged 2.2×10^5 , 9.1×10^4 and 3.8×10^5 cfu g^{-1} of forage DM for Low, Medium and High DM forages, respectively, and total counts were 10^2 lower in Low DM hay than in High DM hay by the end of storage. Generally, total bacterial counts decreased as storage progressed.

Total fungal counts varied ($P < 0.05$) on a daily basis within treatments (Fig. 3). Daily variation in total fungal counts was not influenced ($P > 0.05$) by moisture content or day from day 1 to day 8. Moisture content had an influence ($P < 0.05$) on total fungal counts after day 9, fungal counts being consistently higher in the Low DM forage relative to Medium and High DM forages.

Moisture content at baling had a marginal influence ($P = 0.051$) on the number of fungal species present during the early phase of storage. The highest number of species was observed in the Medium DM forage. There also was high ($P < 0.05$) day-to-day variation in number of species within each treatment (Fig. 4). The dramatic fluctuations in number of species within each treatment occurred during a time when storage temperatures were rising (Fig. 1). The number of species also was influenced ($P < 0.05$) by moisture content at baling during the later phase. Species diversity, as measured by the log series α index, was not influenced ($P > 0.05$) by moisture content at baling (Fig. 5). Species diversity also did not vary ($P > 0.05$) on a daily basis within each treatment in either the early or late storage phase. However, during the later phase, moisture treatment affected the linear component of change in α over time ($P < 0.05$). In Medium DM forage α tended to rise with increased storage time, but α was relatively constant at High and Low DM.

In the early storage phase, the Berger-Parker dominance index was not influenced ($P > 0.05$) by moisture content at baling or by day ($P > 0.05$) within each treatment (Fig. 6). Although dominance during this period was not influenced by moisture content at baling, the predominant species were different (Table 1). Yeasts were predominant in Medium

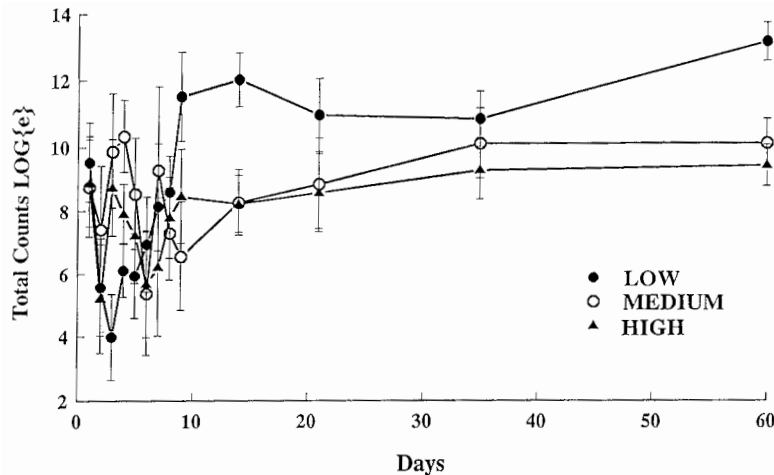


Fig. 3. Total counts of fungi growing in stored alfalfa forage baled at Low (64.1–66.2%), Medium (71.9–73.2%) and High (75.4–77.4%) DM contents.

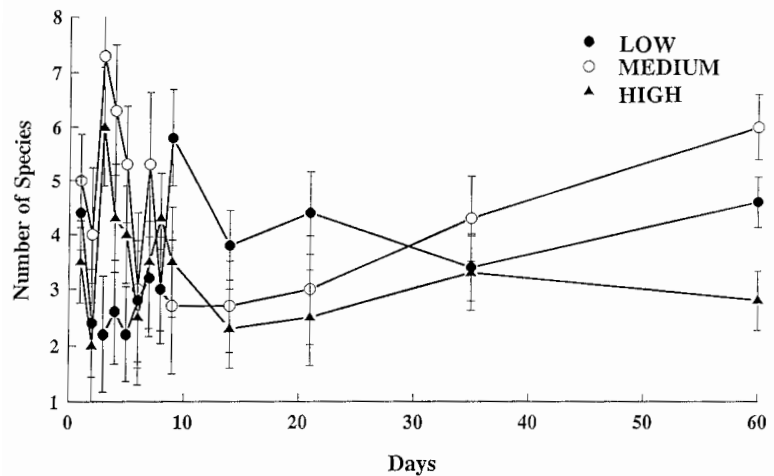


Fig. 4. Number of fungal species detected during the storage for alfalfa forage baled at Low (64.1–66.2%), Medium (71.9–73.2%) and High (75.4–77.4%) DM contents.

and High DM forages, while *Phoma* and *Cladosporium* spp. were predominant in Low DM forage. Storage species appeared much earlier in Low DM forage and this was most probably related to the higher temperature attained.

Species dominance was influenced ($P < 0.05$) by moisture content at baling from day 9 onwards. Species dominance was consistently highest in High DM forage (Fig. 6). *Aspergillus repens* was most often dominant in Medium and High DM forages (Table 1). Unidentified thermotolerant hyphomycetes following either 25 or 40°C incubation were most often dominant in Low DM forage. Thermotolerant hyphomycetes were treated as two different species based on their growth at different temperatures, but because they were not identified, the possibility that they are the same organism can not be ruled out. CA was discussed in detail by Greenacre (1984) and ter Braak (1985). This analysis gives a unique ordination of both the sites of, and the species in, a site \times species matrix. In the context of the current study, “site” represents any one of the three DM treatments on a specific day. The positions of sites and species points with respect to each other show a general relationship. Each species point will lie more or less “in the direction of” the site in which that species is prominent (Greenacre 1984).

The first two axes of the CA accounted for 50% of the variation in the data set representing the entire storage period (Fig. 7a,b). Axis 1 (horizontal in Fig. 1a,b) accounted for 42.1% of the variation and separated early sites of Low, Medium and High DM forages on the positive side of the axis and later sites on the negative side, suggesting that storage time is a major contributor to this axis. The early sites were associated with *Cladosporium*, *Phoma*, *Alternaria* spp. and several yeast species. Therefore, these CA ordination diagrams show a progressive extinction of field species and an increase in storage species with time (Fig. 7b). The later sites were associated with *Aspergillus repens*, *Mucor* spp., *E. nidulans*, *Absidia* spp., *A. flavus* and thermotolerant hyphomycetes. Axis 2 accounted for 7.9% of the variation and was associated with differences between moisture levels (Fig. 7a).

The CA ordination for days 9 to 60 of storage also explained a high (51.6%) percentage of the variation (Fig. 8). Site separation was along the horizontal axis which accounted for 32.1% of the variation. Species associated with Low DM sites were *Absidia* spp., *Mucor* spp., *E. nidulans*, *Aspergillus fumigatus*, unidentified thermotolerant hyphomycetes, and some yeast species (Y3 and Y7). There

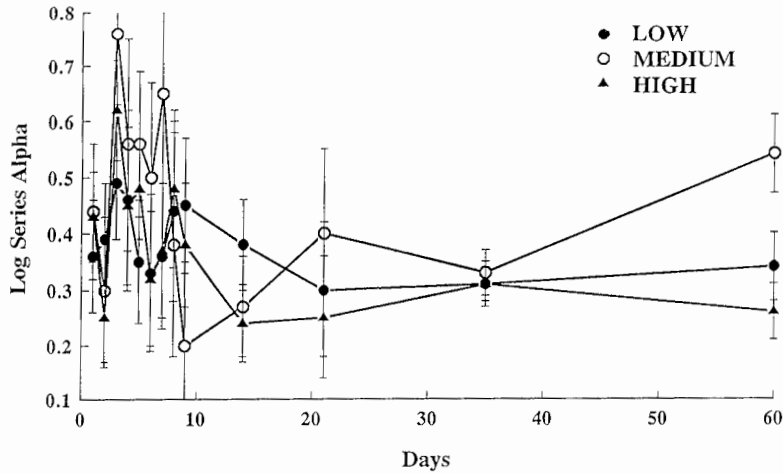


Fig. 5. The log series α diversity index for fungi in alfalfa forage baled at Low (64.1–66.2%), Medium (71.9–73.2%) and High (75.4–77.4%) DM contents.

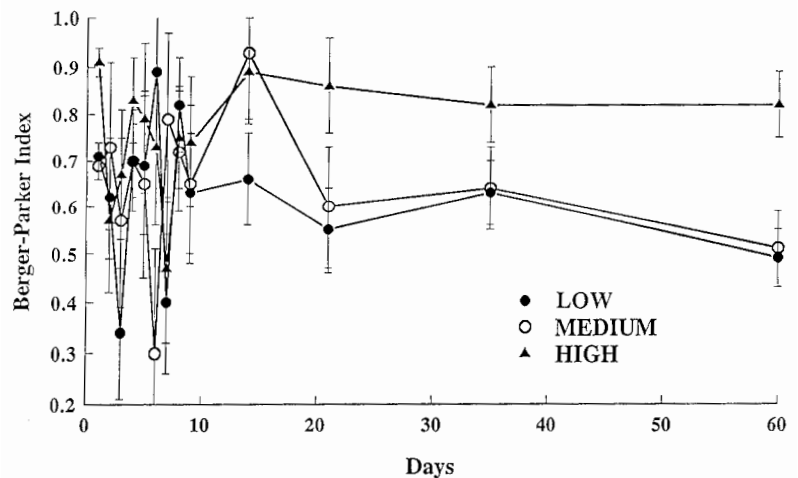


Fig. 6. The Berger-Parker dominance index for fungi in alfalfa forage bales at Low (64.1–66.2%), Medium (71.9–73.2%) and High (75.4–77.4%) DM contents.

was no clear separation of species assemblages between Medium and High DM forage sites, and these sites were associated mainly with *A. repens*, *Phoma* spp. and yeast species (Y2, Y5, and Y8).

Canonical correspondence analysis provides an integrated description of species-environment relationships by assuming a response model that is common to all species, and the existence of a single set of environmental variables to which all species respond (ter Braak 1986; 1987). The resulting ordination diagram shows the pattern of variation in community composition as accounted for by the environmental variables, and also shows approximately the distribution of species along each environmental variable. The CCA can be displayed in an ordination diagram and species and sites are represented by points while environmental variables are represented by arrows (ter Braak 1987). The arrow of an environmental variable points in the direction of maximum change of that variable across the diagram, its length being proportional to the rate of change in this direction.

The CCA ordination diagram for the whole storage period resulted in the environmental variable “day” coinciding with the horizontal axis (Fig. 9). This variable “day”

accounted for 38.1% of the variation in the data set and the resulting ordination supported what was observed with CA analysis. Other environmental variables that were tested in the CCA were crude protein, water-soluble carbohydrate content and total bacteria and they did not contribute additionally to explaining species assemblages.

Moisture content and temperature accounted for 15.8% of the variation in the data set during the later phase of storage, and moisture content accounted for most of the variation (9.9%; Fig. 10). More Low DM sites were situated in positions of higher moisture content and Medium and High DM sites were most often situated where moisture content was relatively lower. Associations of fungal species and sites were similar to those in CA ordinations for the same period.

DISCUSSION

Among the environmental factors involved in determining fungal species succession in stored agricultural products, available moisture, temperature and substrate are the most important (Magan and Lacey 1984a; Pitt and Hocking 1985). Other important environmental factors include pH, gaseous composition of the atmosphere, nutrient availabili-

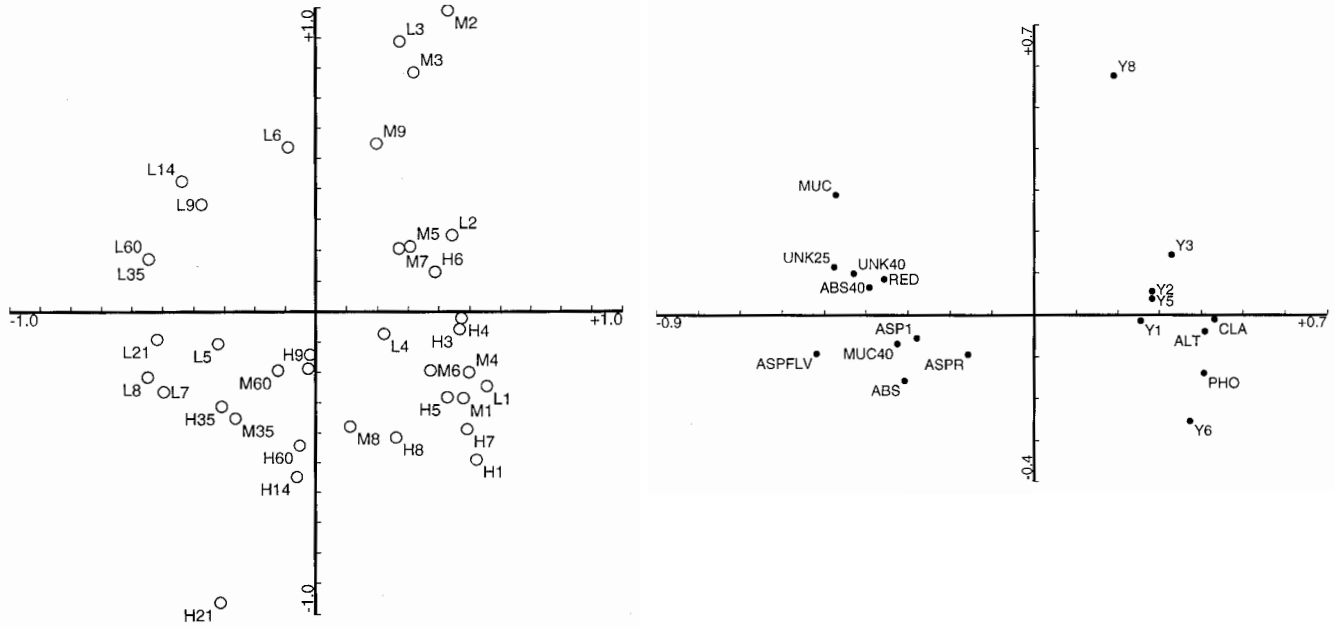


Fig. 7. The CA ordination diagrams (following quadratic detrending) for 60 d of storage showing sites (7a) and fungal species (7b). The fungal species include yeasts which are numbered (Y) 1 to 8; CLA = *Cladosporium* spp., ALT = *Alternaria* spp., PHO = *Phoma* spp., ASPR = *A. repens*, ASPFLV = *A. flavus*, ASP1 = *E. nidulans*, RED = an unidentified species, UNK25 and UNK40 = unidentified thermotolerant hyphomycetes incubated at 25 and 40°C, respectively. ABS and ABS40 = *Absidia* spp. incubated at 25 and 40°C, respectively. MUC and MUC40 = *Mucor* spp. following incubation at 25 and 40°C, respectively. The site numbering represents the forage treatment and day of sampling, e.g. L1 = Low DM forage site on day 1 of storage.

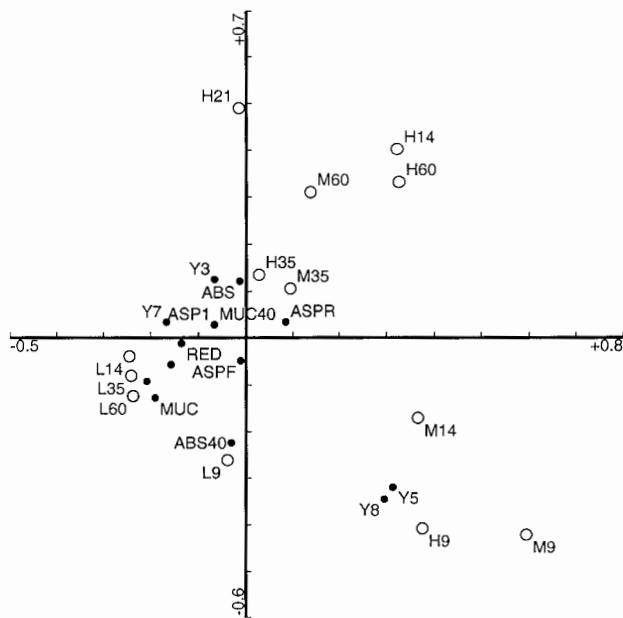


Fig. 8. The CA ordination diagram for days 9 to 60 storage showing sites (○) and fungal species (●). Fungal species and sites are defined as in Fig. 7.

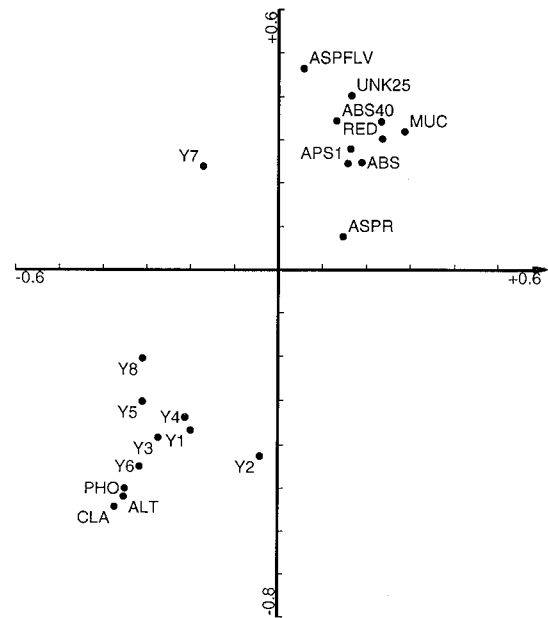


Fig. 9. The CCA ordination diagram for the 60-d storage period with fungal species and day (arrow) as the environmental variable. The day arrow coincided with the horizontal axis. The species are defined as in Fig. 7.

ty, and interaction with other organisms (Magan and Lacey 1984a; Pitt and Hocking 1985). Most fungal species are not affected by pH over a broad range of 3 to 8, and the opti-

num seems to be 5 (Pitt and Hocking 1985). In forage samples collected during storage of the current study, pH averaged 6.5 with no change over time.

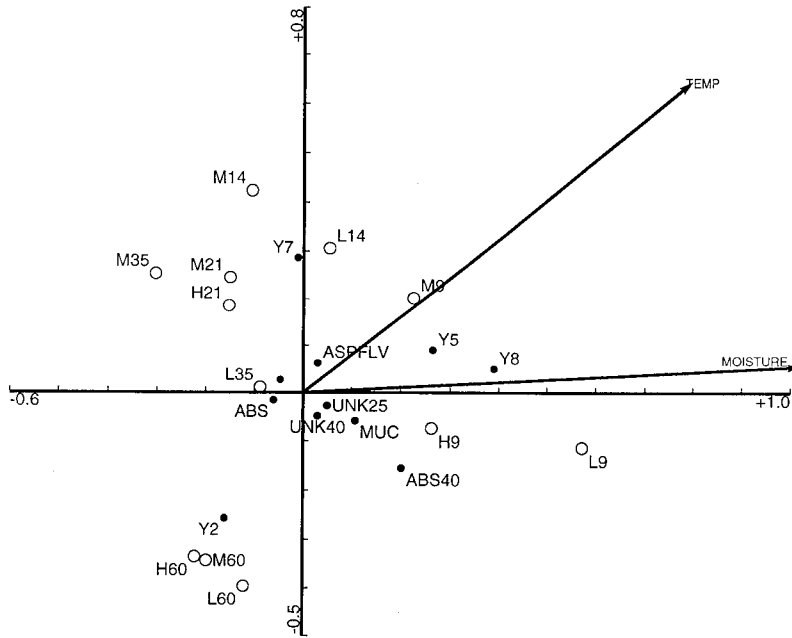


Fig. 10. The CCA ordination diagrams for days 9 to 60 with sites (○), fungal species (●) and environmental variables (arrows). The sites and species are identified as in Fig. 7.

Fungi rarely occur in a monospecific culture in stored products. Rather they are present as a group of interacting species along with bacteria, and often the conditions under which fungi are detected in greatest numbers are those under which they survive or compete best, not at which they grow best (Magan and Lacey 1984b; Lacey 1989). Total bacterial counts, when imposed as an environmental variable, were not important in explaining fungal species assemblages during storage.

The occurrence of fungal species in Low, Medium and High DM forage in the current study can be interpreted with the help of the measured environmental variables, moisture content and temperature. Moisture content and water activity (a_w) are linearly related, and generally, a higher moisture content is associated with a higher a_w . Extrapolating from moisture sorption isotherms for alfalfa stems and leaves constructed by Albert et al. (1989), forages in the current study were stored with initial a_w of approximately 0.93, 0.88 and 0.85 for Low, Medium and High DM forages, respectively. The highest temperatures attained during storage for Low, Medium and High DM forages were 54.5, 50 and 44°C, respectively.

By the time peak temperatures were reached in all forages, the species with optimum temperatures for growth of about 25°C and a_w 0.99–1.00, namely *Cladosporium*, *Alternaria* and *Phoma* spp., had been eliminated. The later storage phase was marked by an appearance of several *Aspergillus* spp., *Mucor* spp., *Absidia* spp. and some yeasts. *Aspergillus* spp. are characteristic colonizers of stored products and, since they differ considerably in their requirements, the predominant species is often a good indicator of storage conditions (Lacey 1989). In Medium and High DM forages, *A. repens* was the dominant fungal species. *Aspergillus repens* thrives in agricultural products stored at

20–25% moisture ($0.90a_w$) which heat to about 35°C, but the optimum for growth is 25–27°C (Lacey 1989). *Emericella nidulans* and *A. fumigatus* were found in Low DM forage and certainly conditions in this forage were favourable for proliferation of these species. Both these species have the ability to grow in high temperature environments up to 50°C for *E. nidulans* and 60–65°C (with an optimum of 40–42°C) for *A. fumigatus*, and high moisture environments, 30–35% (a_w 0.97) for *E. nidulans* and 35–40% (a_w > 0.98) for *A. fumigatus* (Lacey 1989). During the later storage phase there was little difference in temperature and moisture content among forages. Therefore, it appears that peak temperature may be a determining factor in the proliferation of particular dominant species.

Mucor spp. and *Absidia* spp. also occurred to a larger extent in Low DM forage, which might indicate a tolerance for high moisture and temperature. The most dominant fungal species in Low DM forage were unidentified thermotolerant hyphomycetes, but since they were not identified, their ecological requirements cannot be discussed with any certainty. These species seem to thrive in high moisture environments where forage can heat to high temperatures. Yeasts were predominant only in the early storage phase and most were eliminated by day 9 of storage.

CONCLUSIONS

The moisture content of alfalfa at the time of baling was important in determining total fungal counts, number of species present, and degree of species dominance during the later phase (days 9 to 60) of storage. The temporal pattern of species diversity was also affected by moisture content at baling. The most important fungal species after 60 d storage of alfalfa hay baled and stored at Medium and High DM contents (71.9–73.2% and 75.4–77.4%, respectively) were

Aspergillus repens, *Absidia* spp. and yeasts. The main fungal species observed by day 60 of storage were *E. nidulans*, *A. fumigatus*, *Absidia* spp. and thermotolerant hyphomycetes. In hay baled at 64.1–66.2% DM (Low DM), moisture content influences temperature during early storage, and temperature during the early storage period likely influences fungal species and succession in the later phase of storage. More stable conditions, in terms of fungal counts and number of species, were found only after temperatures had peaked and were beginning to decrease in all forage treatments.

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Albert, R. A., Huebner, B. and Davis, L. W. 1989. Role of water activity in the spoilage of alfalfa hay. *J. Dairy Sci.* **72**: 2573–2581.

Berger, W. H. and Parker, F. L. 1970. Diversity of planktonic Foraminifera in deep sea sediments. *Science* **168**: 1345–1347.

Breton, A. and Zwaenepoel, P. 1991. Succession of moist hay microflora during storage. *Can. J. Microbiol.* **37**: 248–251.

Clevstrom, G. and Ljunggren, H. 1984. Occurrence of storage fungi, especially aflatoxin-forming *Aspergillus flavus*, in soil, greenstuff and prepared hay. *J. Stored Prod. Res.* **20**: 71–82.

Fisher, R. A., Corbet, A. S. and Williams, C. B. 1943. The relationship between the number of species and the number of samples in a random sample of an animal population. *J. Anim. Ecol.* **12**: 42–58.

Greenacre, M. J. 1984. Theory and applications of correspondence analysis. Academic Press, Inc., London, UK.

Gregory, P. H., Lacey, M. E., Festenstein, G. N. and Skinner, F. A. 1963. Microbial and biochemical changes during the moulding of hay. *J. Gen. Microbiol.* **33**: 147–174.

Kaspersson, A., Hlodversson, R., Palmgren, V. and Lindgren, S. 1984. Microbial and biochemical changes occurring during deterioration of hay and preservative effect of urea. *Swed. J. Agric. Res.* **14**: 127–133.

King, A. D., Hocking, A. D. and Pitt, J. I. 1979. Dichloran-Rose Bengal medium for enumeration and isolation of molds from foods. *Appl. Environ. Microbiol.* **37**: 959–964.

Knudtson, W. U. and Kirkbride, C. A. 1992. Fungi associated with bovine abortion in the northern plains states (USA). *J. Vet. Diagn. Invest.* **4**: 181–185.

Lacey, J. 1975. Potential hazards to animals and man from microorganisms in fodder and grain. *Trans. Br. Mycol. Soc.* **65**: 171–184.

Lacey, J. 1989. Pre- and Post-harvest ecology of fungi causing spoilage of foods and other stored products. *J. Appl. Bacteriol. Symposium Suppl.* 11s–25s.

MacFaddin, J. R. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. J. Butler, ed. Williams & Williams, Baltimore, MD.

Magan, N. and Lacey, J. 1984a. Effect of temperature and pH on water relations of field and storage fungi. *Trans. Br. Mycol. Soc.* **82**: 71–81.

Magan, N. and Lacey, J. 1984b. Effect of water activity, temperature and substrate on interactions between field and storage fungi. *Trans. Br. Mycol. Soc.* **82**: 83–93.

Pitt, J. I. and Hocking, A. D. 1985. Fungi and food spoilage. Academic Press, Inc., North Ryde, Australia.

Pizarro, E. A. and Warboys, I. B. 1980. The effect of the wilting period on the microflora of harvested pasture plants. Pages 221–223. *in* Forage conservation in the 80's. Occasional symposium no. 11. British Grassland Society, Brighton, UK.

SAS Institute, Inc. 1985. SAS user's guide: Statistics. 6th. edition. SAS Institute, Inc., Cary, NC.

Statistics Canada. 1992. Agriculture Division. Agriculture profile of Canada — Part 1. Industry, Science and Technology. Census of Agriculture Catalogue #93-350, Ottawa, ON.

Taylor, L. R., Kempton, R. A. and Woiwood, I. P. 1976. Diversity statistics and the log series model. *J. Anim. Ecol.* **45**: 255–271.

ter Braak, C. J. F. 1985. Correspondence analysis of incidence and abundance data: Properties in terms of a unimodal response model. *Biometrics* **41**: 859–873.

ter Braak, C. J. F. 1986. Canonical correspondence analysis: A new eigenvector technique for multivariate direct gradient analysis. *Ecol.* **67**(5): 1167–1179.

ter Braak, C. J. F. 1987. The analysis of vegetation-environment relationships by canonical correspondence analysis. *Vegetatio* **69**: 69–77.

ter Braak, C. J. F. 1987–1992. CANOCO: A Fortran program for canonical correspondence analysis and detrended correspondence analysis. IWIS-TNO, Wageningen, The Netherlands.

Wilkinson, L. 1988. Systat: The system for statistics. Systat Inc., Evanston, IL.

Williams, C. B. 1964. Patterns in the balance of nature and related problems in quantitative ecology. Academic Press Inc., London, UK.

Wittenberg, K. M. 1994. Nutritive value of high moisture alfalfa hay preserved with *Pediococcus pentosaceus*. *Can. J. Anim. Sci.* **74**: 229–234.