

# EFFECT OF MACERATION ON RESPIRATION OF ALFALFA

T. J. Kraus, R. E. Muck, R. G. Koegel

**ABSTRACT.** *Unconditioned and macerated alfalfa samples at approximately 55 and 80% moisture wet basis (w.b.) were incubated at 11 and 31°C for 48 h and rates of CO<sub>2</sub> respired from each sample were measured. Production of CO<sub>2</sub> from unconditioned alfalfa samples appeared to be due to plant cell respiration while production of CO<sub>2</sub> from the macerated alfalfa samples appeared to be due primarily to microbial growth. Typically, the initial respiration rates of the macerated samples were less than the control samples. When the samples were incubated at 31°C, the rates of CO<sub>2</sub> production from the macerated samples increased exponentially to approximately four times that of the control samples 6 to 20 h after the samples had been macerated. Consequently, the cumulative loss of DM from the macerated samples during the first 12 h of incubation was less than that of the control samples; however, after 12 h of incubation, loss of DM from the macerated samples increased to nearly twice the level of the control samples. At 11°C, DM losses over 48 h in the macerated treatments were lower than the corresponding control treatments in three of four cases.*

**Keywords.** *Alfalfa, Forage, Maceration, Respiration, Forage conditioning.*

**M**aceration and the formation of thin continuous mats of forage is an emerging technology in the field of forage conditioning. Maceration is a process in which plant herbage is severely conditioned, and the leaves and upper stem fragments are mashed and/or pureed. Once conditioned, the forage is pressed into a thin continuous mat and placed on top of the field stubble to dry. Under favorable drying conditions, this new process allowed forage to dry three times as fast as conventionally conditioned forage (Shinners et al., 1987); which may reduce losses due to leaching of nutrients caused by rain and reduce the loss of carbohydrates due to respiration. However, studies have found that mechanical conditioning of plant herbage can increase the rate of respiration leading to increased loss of carbohydrate.

Some of the earliest work in this field was done by Johnstone (1925) who found the respiration rate of sweet potatoes having exposed injuries was 97% greater than that of uninjured sweet potatoes. Audus (1935) found the rate of respiration of cherry laurel leaves, as measured by CO<sub>2</sub> production, increased to twice the normal rate as a result of mere deformation due to bending and rubbing.

Hill et al. (1959) measured the respiration rates of gladiolus leaves after subsection to different types of injury.

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Manufacturer's names are given for identification only and do not imply recommendation.

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They found the respiration rates in the vicinity of the damaged area on leaves crushed between a hammer and a board were 23% greater than uncrushed leaves. Simpson (1961) found that crushing white clover, alfalfa, and short rotation ryegrass caused a stimulation of respiration; the degree of stimulation often being greater with a heavy crush than a light crush.

Rotz et al. (1990) compared the respiration losses of field cured mats of macerated alfalfa and conventionally conditioned alfalfa. They found no significant difference in total respiration losses between the two treatments. Rotz et al. (1990) suggested this was due to faster drying of the matted alfalfa under the favorable drying conditions in this study. If the drying conditions had been less favorable, however, the results may have been different.

The key to reducing respiration losses is to remove the water within the plant tissue as quickly as possible. Maceration abrades the waxy cutin layer of the plant's epidermis and ruptures numerous cells thus reducing the resistance of moisture movement from the plant herbage to the surrounding environment. It is believed, however, that mechanically rupturing cells destroys boundaries between various organelles liberating enzymes which break down stored carbohydrates and proteins. Moreover, mechanically rupturing cell walls makes cell solubles readily available to microorganisms, enabling them to reproduce at accelerated rates. Under unfavorable drying conditions, as when a crop remains in the field overnight, these organisms may consume significant amounts of carbohydrate.

Therefore, the objective of this research was to determine the effect of forage moisture content, maceration level, and temperature on CO<sub>2</sub> production.

## PROCEDURE

Second cutting alfalfa was harvested at the University of Wisconsin West Madison Experimental Station in Madison, Wisconsin. The alfalfa was divided into two portions. One

portion was not conditioned, the other was severely conditioned using a rotary impact macerator (Kraus, 1997). This unit had several blunt blades attached to a high speed electric motor which was mounted inside a vertical cylindrical tube. As plant material was dropped through the tube, it was struck or impacted numerous times by the blunt blades causing the plant herbage to be extremely macerated.

A conditioning index (CI) developed by Kraus et al. (1997) was used to quantify the conditioning level of each treatment. This method is based upon measuring the amount of electrolyte leached from ruptured cells. Ten samples from each treatment were measured. The average CI of the unconditioned and severely macerated treatments were 2% and 90%, respectively.

Figure 1 is a schematic of the apparatus used to measure respiration rates. The apparatus had 6 components: a tank of compressed dry air, an airflow regulator, a respiration chamber, a desiccant, a gas mass flow meter, and a mass spectrometer. Four respiration chambers were fabricated allowing simultaneous measurement of multiple samples.

Each chamber was made of PVC plastic pipe 61.0 cm long with inner and outer diameters of 14.6 and 15.3 cm, respectively. Each chamber had a removable cap and multiple perforated shelves spaced 15.3 cm apart. Samples of macerated and unconditioned alfalfa were placed on the shelves in a loose structure (i.e., the macerated samples were not pressed into a mat-type structure). The shelves allowed each sample to be subdivided to prevent the samples from compacting under their own weight and allowed air to flow uniformly through the entire cross-section of each sample.

To determine the relationship between level of conditioning and rate of respiration at two moisture levels, one sample of each treatment was oven dried at 50°C to approximately 50% moisture, w.b. Once dried, these samples were refrigerated at 5°C for approximately 48 h to suppress microbial growth and plant respiration until the rate of respiration could be measured.

Simultaneously, four 1000 g samples of fresh material (two samples from each treatment) were placed into four respiration chambers. Two respiration chambers, one for each treatment, were placed into a convection type environmental chamber at 11°C and the other two respiration chambers were placed into a convection type environmental chamber at 31°C.

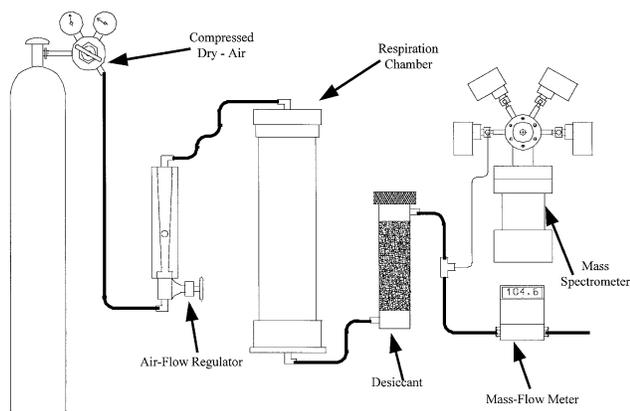


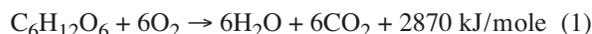
Figure 1—Respiration apparatus.

Dry air was metered into the top of each respiration chamber at approximately 300 mL/min. The air exited the bottom of each chamber and passed through an anhydrous calcium sulfate desiccant to remove moisture evaporated from the sample. After the air passed through the desiccant, the air mass flow rate and gas composition were measured at 15-min intervals for approximately 48 h using a FMA-5000 series mass flow meter and an AMETEK System 1000 mass spectrometer, respectively.

It was estimated that an airflow rate of 300 mL/min would limit the CO<sub>2</sub> concentration to less than 3% (Rotz et al., 1989). Based on a model developed by Pitt et al. (1985), aerobic microbial activity was not retarded in environments having CO<sub>2</sub> concentrations of 3% or less. In addition, it was estimated that sample moisture would remain nearly constant throughout the 48-h incubation period because an airflow rate of 300 mL/min limited the loss of moisture from each sample.

At the end of the 48-h period, the samples were removed from the respiration chambers, and a previously prepared 500 g sample of alfalfa at 50% moisture from each treatment was placed into two of the four chambers. The gas composition and the mass flow rate were measured using the procedures previously described. This experiment was replicated twice. The gas composition of the compressed dry air within the gas cylinder was measured prior to each experiment.

Plant respiration is considered as the complete oxidation of hexose sugar to carbon dioxide and water (Rees, 1982). The stoichiometric relationship is expressed as:



This stoichiometric expression was used to calculate the mass of dry matter consumed per unit mass of sample by multiplying the stoichiometric ratio by the total mass of CO<sub>2</sub> produced. Several studies have found good agreement between the theoretical and measured values of DM loss, CO<sub>2</sub> production, and O<sub>2</sub> uptake from respiration (Greenhill, 1959; Melvin and Simpson, 1963; Simpson, 1961).

Table 1. Initial and final moisture levels of macerated and control alfalfa samples incubated for approximately 48 h at 31 and 11°C

|         |                  | Initial Moisture (%) | Final Moisture (%) |
|---------|------------------|----------------------|--------------------|
| Trial 1 | Macerated (31°C) | 78.9                 | 79.7               |
|         | Control (31°C)   | 78.9                 | 81.1               |
|         | Macerated (11°C) | 78.9                 | 78.3               |
|         | Control (11°C)   | 78.9                 | 79.0               |
|         | Macerated (31°C) | 49.2                 | 49.1               |
|         | Control (31°C)   | 55.2                 | 53.3               |
|         | Macerated (11°C) | 49.2                 | 50.1               |
|         | Control (11°C)   | 55.2                 | 52.9               |
| Trial 2 | Macerated (31°C) | 78.2                 | 79.9               |
|         | Control (31°C)   | 78.2                 | 77.5               |
|         | Macerated (11°C) | 78.2                 | 78.0               |
|         | Control (11°C)   | 78.2                 | 77.8               |
|         | Macerated (31°C) | 56.2                 | 60.3               |
|         | Control (31°C)   | 60.6                 | 59.0               |
|         | Macerated (11°C) | 56.2                 | 59.9               |
|         | Control (11°C)   | 60.6                 | 59.2               |

## RESULTS AND DISCUSSION

Initial and final moisture contents of each sample were approximately equal (table 1). Apparently, the moisture lost from the gas flow through the chambers was similar to the amount of water produced by respiration.

Figures 2 and 3 are plots of CO<sub>2</sub> production rate vs. incubation time of each treatment at 31°C for trials 1 and 2, respectively. Both figures illustrate that the respiration rates of the control samples at moisture levels of 78 and 79% w.b. decreased throughout the 48 h incubation period from approximately 1.0 to 0.4 g CO<sub>2</sub>/h.

The initial respiration rates of the high moisture control samples were approximately four times that of the dried control samples. Both figures illustrate that the initial respiration rates of the control samples at moisture levels of 55 and 61% w.b. remained nearly constant for the first 24 to 36 h of the incubation period at approximately 0.25 g CO<sub>2</sub>/h before rising in the latter hours.

The respiration rates of the macerated samples varied throughout the 48-h incubation period. In general, the initial respiration rates of the macerated samples were lower than the control samples at equivalent moisture levels. However, several hours after the samples had been macerated the respiration rates increased rapidly to more than four times that of the control samples.

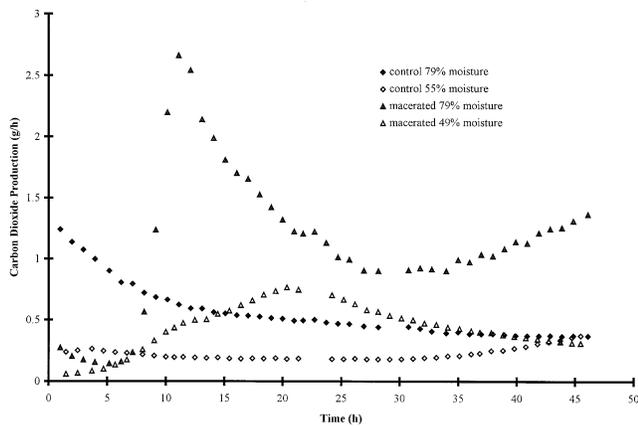


Figure 2—Carbon dioxide production vs incubation time of each treatment at 31°C. Trial 1.

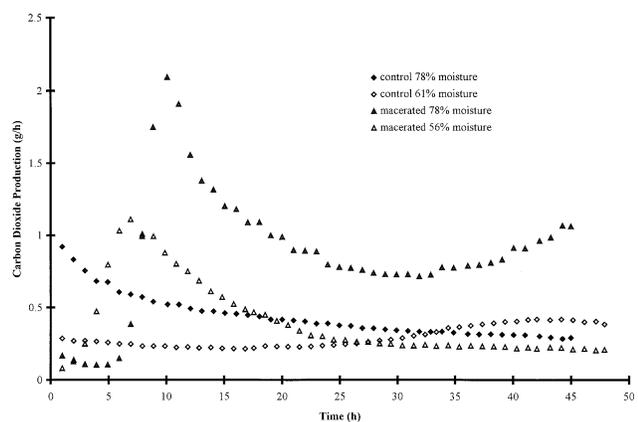


Figure 3—Carbon dioxide production vs incubation time of each treatment at 31°C. Trial 2.

Figures 2 and 3 illustrate that the initial respiration rates of the macerated samples at moisture levels of 78 and 79% w.b. were less than 0.25 g CO<sub>2</sub>/h for approximately 6 h. Between 6 and 12 h, these rates increased to approximately 2.5 g CO<sub>2</sub>/h. Between 12 and 30 h the respiration rates decreased to approximately 0.8 g CO<sub>2</sub>/h and then gradually increased for the remainder of the incubation period.

Trends of the dried macerated alfalfa samples were similar to those of the higher moisture macerated samples. It can be seen in figure 3 that the initial respiration rate of the macerated sample at 56% moisture w.b. was approximately 0.1 g CO<sub>2</sub>/h. Although the lag time of this sample was relatively short, the rate at which CO<sub>2</sub> production increased was similar to that of the macerated samples at moisture levels of 78 and 79% w.b.

Similarly, figure 2 illustrates that the initial respiration rate of the macerated sample at 49% moisture w.b. was less than 0.2 g CO<sub>2</sub>/h for approximately 6 h. Although the respiration rate did not increase as rapidly as that of the other macerated samples, it did increase to approximately 0.9 g CO<sub>2</sub>/h between 6 and 20 h of incubation.

Figures 4 and 5 are plots of CO<sub>2</sub> production rate versus incubation time of each treatment at 11°C for trials 1 and 2, respectively. It can be seen in figures 4 and 5 that the initial respiration rates of the control samples at moisture levels of 79 and 78% w.b. were approximately 0.6 and 0.4 g

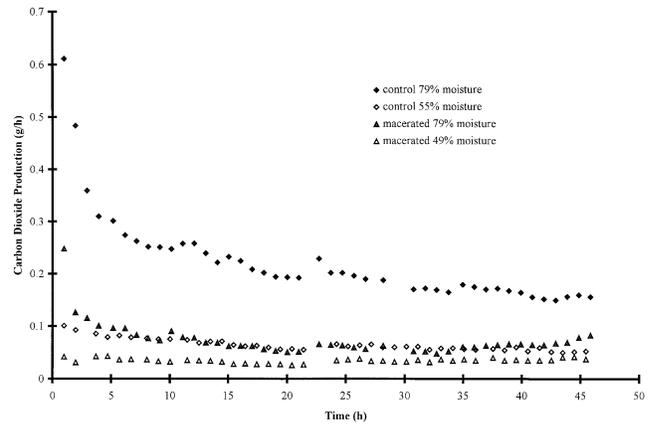


Figure 4—Carbon dioxide production vs incubation time of each treatment at 11°C. Trial 1.

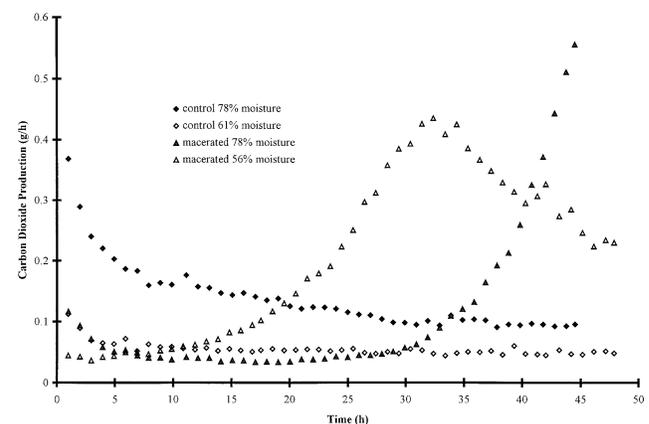


Figure 5—Carbon dioxide production vs incubation time of each treatment at 11°C. Trial 2.

CO<sub>2</sub>/h, respectively. Lowering the temperature from 31 to 11°C reduced the initial respiration rates of the control samples by approximately 50%.

The trends of the control samples incubated at 11°C were similar to those of the samples incubated at 31°C. Both of these figures illustrate that the respiration rates of the control samples at moisture levels of 78 and 79% w.b. decreased throughout the 48-h incubation period. In both experiments, the rates of CO<sub>2</sub> production decreased from approximately 0.5 to 0.1 g/h.

The initial respiration rates of the high moisture control samples were approximately four times greater than the low moisture control samples. It can be seen in both figures that the initial respiration rates of the control samples at moisture levels of 55 and 61% w.b. remained nearly constant throughout the 48-h incubation period at less than 0.1 g CO<sub>2</sub>/h.

Both figures 4 and 5 illustrate that the initial respiration rates of the macerated samples were lower than the control samples at equivalent moisture levels. The respiration rate of both macerated samples in trial 1 (fig. 4) remained nearly constant throughout the entire 48-h incubation period. In the second trial (fig. 5) respiration rates from both macerated samples increased after relatively long lag times.

The initial respiration rate of the control material at each moisture level and temperature was compared to results from empirical models developed by Rotz (1995) and Pitt et al. (1985). Figure 6 illustrates that there was a relatively good correlation between the predicted and measured values. The model developed by Rotz (1995) underestimated the initial respiration rates by approximately 25% and the model developed by Pitt et al. (1985) overestimated the initial respiration rates by nearly 87%.

What caused the rate of respiration of the control samples to decrease throughout the 48-h incubation period and that of the macerated samples to vary throughout the 48-h incubation period? When a plant is severed from its roots, the cells in the upper portion of the plant continue to live and to consume stored carbohydrates. When these sources of energy are depleted or the plant is desiccated, the cells die.

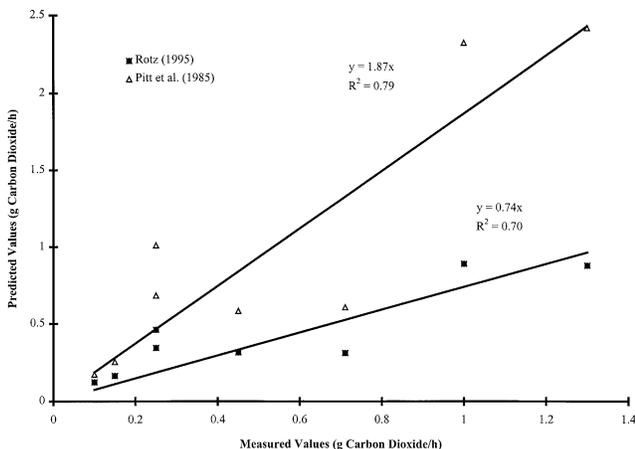


Figure 6—Predicted respiration rates by Rotz (1995) and Pitt et al. (1985) vs measured initial respiration rates of control samples.

When the plant material is severely conditioned, many plant cell walls are broken, thereby killing the cells. Because these cells are dead, one would expect no respiration. This explains why the initial respiration rates of the macerated samples were less than those of the control samples at equivalent moisture levels. However, when plant cells are ruptured, much of the plant sap within the cells is liberated and readily available to microorganisms that consume sugars and other compounds in the sap. Because of the availability of sugars, these microorganisms may multiply exponentially, which may explain why the respiration rates of the macerated samples increased rapidly after the initial lag time.

The decrease in respiration rates of the macerated samples after 5 to 10 h may have occurred for two reasons. One is that the microorganisms continued to multiply until they consumed the most readily available sugars, and when these sources of energy were depleted the microorganisms died, bringing about a decrease in rate of CO<sub>2</sub> production. Alternately, the microorganisms may have created an inhibitory environment causing respiration rates to decrease. The second increase in CO<sub>2</sub> production may indicate that different microorganisms began to multiply. This succession of microorganisms has been observed in the heating and spoilage of silage (Muck and Pitt, 1994).

Thus, for the control samples, CO<sub>2</sub> production appears to have been primarily due to plant respiration of stored carbohydrate. As cells died, the respiration rate decreased. For the macerated samples, CO<sub>2</sub> production appears to have been primarily due to aerobic microbial growth. As microorganisms multiplied, respiration rates increased; when they died, respiration decreased.

Dry matter loss due to respiration was calculated assuming 0.68 C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> lost/g CO<sub>2</sub> produced. Figures 7 and 8 are plots of cumulative DM loss as a fraction of the initial DM content versus incubation time of each treatment at 31°C for trials 1 and 2, respectively. Typically, for incubation times less than 12 h the cumulative DM losses from the macerated samples were less than those of the control samples at equivalent moisture levels. For incubation times greater than 12 h, the cumulative DM losses from the macerated samples increased rapidly to a level approximately two times that of the control samples at equivalent moisture levels.

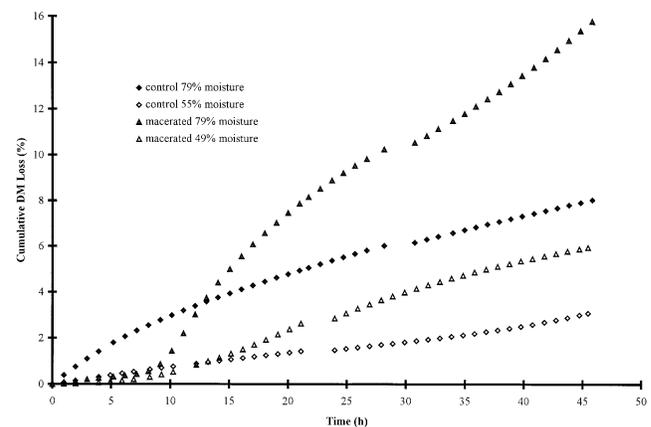


Figure 7—Cumulative DM loss vs incubation time of each treatment at 31°C. Trial 1.

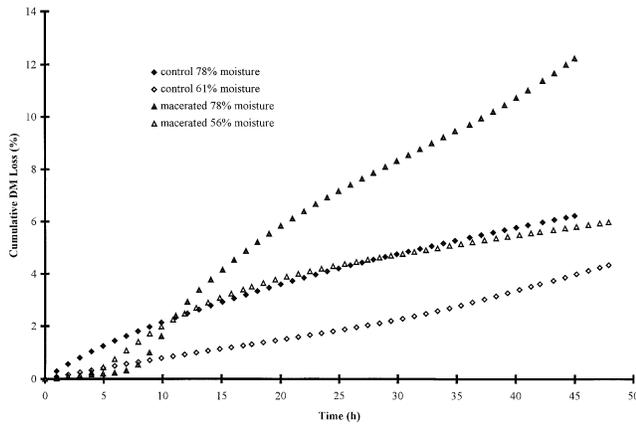


Figure 8—Cumulative DM loss vs incubation time of each treatment at 31°C. Trial 2.

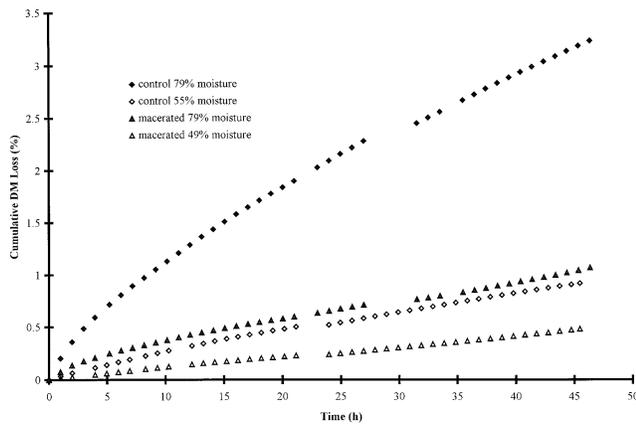


Figure 9—Cumulative DM loss vs incubation time of each treatment at 11°C. Trial 1.

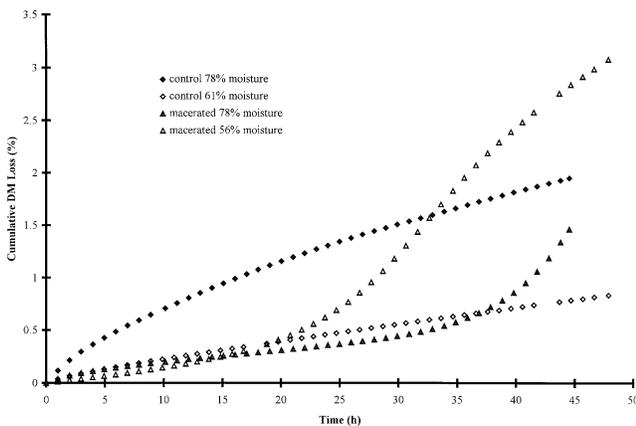


Figure 10—Cumulative DM loss vs incubation time of each treatment at 11°C. Trial 2.

Figures 9 and 10 are plots of cumulative DM loss over incubation time for each treatment at 11°C for trials 1 and 2, respectively. At this temperature, loss of DM due to respiration was less than 3% for all treatments. At this temperature, the DM losses over 48 h in the macerated samples were lower than the corresponding control treatments in three of four cases.

These results indicate that under good drying conditions, loss of carbohydrates from macerated forage can be less than that from unconditioned forage. However, under poor drying conditions, particularly when macerated forage at a relatively high moisture level remained in a warm aerobic environment for more than 12 h, loss of carbohydrates was significantly greater than that from unconditioned forage. This suggests that harvesting practices may have to be modified depending on the level at which forage is conditioned. Many times, a standing crop is cut during the evening and remains in the field overnight. This practice should be avoided under hot, humid climate conditions when the forage is macerated because significant loss of carbohydrate can occur due to accelerated aerobic microbial growth. However, under cooler climate conditions, microbial growth is suppressed therefore, loss of carbohydrate should be less than or equal to conventionally conditioned forage.

It should be noted that Rotz et al. (1990) found no significant difference between total respiration losses of macerated and conventionally conditioned forages; whereas, in a later study (Rotz et al., 1991) found that macerated alfalfa produced lower respiration losses under rapid drying conditions. Our results indicate that both of these scenarios are possible. Figures 7 and 8 illustrate that DM losses between the macerated and control samples were typically the same at approximately 11 h of incubation. Initially, DM losses of the macerated alfalfa were lower than the control at 31°C but apparent microbial respiration in the macerated forage caused an increase in DM losses later during the incubation period. It stands to reason that the predominate biological processes that occur would be dependent upon the level of conditioning. Currently, the empirical models developed to predict loss of DM due to respiration do not account for level of conditioning. This research clearly indicates that applying these empirical models to different harvesting systems could lead to significant error under different drying conditions. Further research should be conducted on conditioning level and its effect on respiration losses under various drying conditions.

## SUMMARY

Production of CO<sub>2</sub> from unconditioned alfalfa samples appeared to be due primarily to plant cell respiration while CO<sub>2</sub> production from the macerated alfalfa samples, particularly at 31°C appeared to be due primarily to microbial growth.

The rates of CO<sub>2</sub> production from the unconditioned alfalfa samples decreased throughout the 48-h incubation period. The rates of CO<sub>2</sub> production from the macerated samples varied throughout the 48-h incubation period.

Typically, the initial respiration rate of the macerated alfalfa samples were less than those from the unconditioned alfalfa samples at equivalent moisture levels. When the samples were incubated at 31°C, the rates of CO<sub>2</sub> production from the macerated samples increased rapidly to approximately four times that of the control samples to approximately 12 h after the samples had been macerated. Consequently, the cumulative loss of DM from the macerated samples during the first 12 h of incubation was less than that of the control samples at equivalent

moisture levels. However, after 12 h of incubation, cumulative loss of DM from the macerated samples increased to nearly twice the level of the control samples.

At 11°C, loss of DM due to respiration was typically less than 3% for all treatments. At this temperature, the DM losses over 48 h in the macerated samples were lower than the corresponding control treatments in three of four cases.

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