# YIEDS OF ALFALFA VARIETIES WITH DIFFERENT FALL DORMANCY LEVELS IN NORTHEAST CHINA

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### Abstract

Fall dormancy (FD) is an important indicator of winter hardiness in alfalfa (*Medicago sativa* L.), the relationship between FD and the yield potential of alfalfa varieties were investigated to survey annual dry matter (DM) yields with FD levels in the northeast regions with cold winters. During three consecutive years, all varieties of five FD levels survived over the winter without any persistency problems and there were no differences in annual DM yields of varieties among FD levels. Among the same FD varieties, DM yields for some of the dormant, semi-dormant and non-dormant varieties were found no correlated with FD levels. In conclusion, it suggests that different FD levels no effected on yields of alfalfa in the cold regions, such as North-east China.

### Introduction

Alfalfa (Medicago sativa L.) is an important crop in the world, which has been planted for over 2,000 years in China (Li et al., 2010). However, alfalfa varieties developed from different geographic regions have distinct characteristics.Fall dormancy (FD) is defined as reduced growth during the fall that comes with reducing day length and temperature (Malinowski et al., 2007), and often employed to assess cultivar differences in winter tolerance (Volenec et al. 2002). FD plays an important role in variety adaptation to particular regions associated with winter survival, and it is classified into three groups: dormant (FD 1-3), semi-fall dormant (FD 4-6), and non-dormant cultivars (FD>6) (Barnes et al., 1979). It is well known that dormant cultivars produce short and prostrate shoots in autumn, exhibit slow stem elongation after summer harvest, and possess high winter hardiness (Dhont et al., 2002; Haagenson et al., 2003b). In contrast, non-dormant cultivars grow vigorously in autumn, forming long erect shoots, and resume rapid shoot elongation after cutting in summer and autumn (Brummer et al., 2000; Haagenson et al., 2003a).

Due to the importance of FD in alfalfa adaptation and productivity, FD level is often used as the first index of selecting alfalfa varieties (Fairey et al., 1996). Especially fast development in animal husbandry, many alfalfa varieties have been introduced into China from the United States. The concept of FD level was also accepted by the Chinese scientific community. So a new variety must be determined before its approval and characterization of FD levels has become the first criteria in alfalfa variety of China. However, much research has been conducted in the United States (Cunningham et al., 2001; Haagenson et al., 2003a, 2003b), and few in China. There were a few studies reporting on DM yield differences among limited varieties with different FD levels (Zang et al., 2005), a possible relationship between FD and autumn herbage yield Leep et al. (2001), and association between FD and the nonstructural carbohydrates accumulation in alfalfa roots and shoot in the spring (Dhont et al., 2002; Xie et al., 2013; Mukhtar et al., 2013). While some studies show the importance of early growth in production of alfalfa (Wang et al., 2004, 2005), there has been no attempt to establish the link between FD levels and annual DM yields.

Our objectives in this study were determine DM yield differences among five FD levels of 17 foreign-originated varieties in comparison with a local variety, assess if there was a quantitative relationship between annual DM yields and FD levels, and if there were differences in DM yield among varieties of the same FD level.

### **Materials and Methods**

**Experimental conditions:** The field experiments were carried out at the Frigid Forage Research Station located in Suihua region. The Research Station has an altitude of 160m, longitude of 125°58', and latitude of 46°32' N in North-east China. The climate is classifed as a typical chillness semi-wetness monsoon environment and the climate variables (average temperatures and rainfall) were recorded as average monthly data in Table 1.

**Experimental design:** 17 varieties introduced from overseas varieties while the only local variety 'Zhaodong' were conducted by a randomized complete block design with four replications for three consecutive years (2009, 2010, and 2011). FD levels were ranged from 2 to 6 for each variety in Table 2. Each plot is 3 meter long and 2 meter wide with inter-row spacing of 15cm, seeded by hand on 1 May 2008 uniformly. Seeding rate was 15 kg ha<sup>-1</sup>. After seeding, the plot surface was pressed using a corrugated roller. Open perimeter area outside of the experiment was reserved to protect the experimental rows from interferential damage. No fertilizer or irrigation was applied during the experimental periods. Plots were hand-weeded during the growing period whenever necessary for proper weed control.

**Investigation of survival rate and DM yields:** The overwinter survival rate was calculated depending on number of plants (in early November 2008, and again in later April 2009) in one randomly selected row per plot, which were counted according to the following formula:

Survival rate (%) =  $\frac{\text{(plant numbers in April 2009)}}{\text{(plant numbers in November 2008)}} \times 100$ 

	-	-											
Years	Climate variables	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
2008	Average temperature	-21	-20	-18	-13	-8	5	15	20	17	10	-5	-15
	Rainfall	-	-	-	-	22	50.6	110.6	156.4	84.5	-	-	-
2009	Average temperature	-20.5	-19.5	-17.5	-12.5	-7.5	5.5	15.5	20.5	17.5	10.5	-4.5	-14.5
	Rainfall	-	-	-	-	26	63.2	123.2	169	97.1	-	-	-
2010	Average temperature	-20	-19	-17	-12	-7	6	16	21	18	11	-4	-14
	Rainfall	-	-	-	-	24	62.1	122.1	167.9	96	-	-	-
2011	Average temperature	-19.5	-18.5	-16.5	-11.5	-6.5	6.5	16.5	21.5	18.5	11.5	-3.5	-13.5
	Rainfall	-	-	-	-	25.6	56.1	116.1	161.9	90	-	-	-

Table 1. Monthly average temperatures and rainfall for 2008 and 2011in North-east, China.

 Table 2. Variety name, source of origin, their fall-dormancy (FD) levels, and overall average annual dry matter yield of alfalfa used in the field experiment conducted in North-east, China from 2008 to 2011.

Varieties	Breeding country	FD level†	Average yield (Mg ha <sup>-1</sup> yr <sup>-1</sup> )
CW201	United States	2	6.87b
Runner	United States	2	7.94a
WL-252HQ	Canada	2	6.78b
CW300	United States	2	6.68b
Zhaodong	China	2	5.41e
CW301	United States	3	4.95f
WL-323HQ	United States	3	6.48c
Alfaking	United States	3	7.09b
WL-323	United States	4	5.47d
WL-323ML	United States	4	7.62a
Goldenkey	United States	4	7.66a
Durango	Canada	5	6.12cd
Defi	Canada	5	4.63f
Derby	Australia	5	5.47e
Sitel	France	5	6.12c
Sanditi	France	5	6.29c
CW675	United States	6	5.73d
WL-414	United States	6	6.35e

<sup>†</sup>FD level is calculated according to the criterion of Barnes *et al.*, (1979)

The alfalfa plants usually started to grow at near the end of April each year. DM yield of each variety was cut at early blooming stage and was determined for each plot with three cuts per year. Cutting frequencies occurred during a 120-day period of each year where the first cut took place in late May to early June and the third cut at the mid- to the end of July. The final cut for all varieties took place in mid-October each year. During experimental period there were 3 shoot regrowth cycles for the 18 varieties differed in FD levels from 2 to 6, but the average difference in blossom occurrence within a year was only 2 d with extremes of up to 4 d. At each sampling, plants from a 2 by 2 m area in each plot were cut at approximately 5 cm above the ground. After cut, a 300-g sample of green herbage was collected from each plot at each harvest to weigh, and then oven-dry at 65°C to determine DM concentration.

**Statistical analysis:** Because FD level and variety are nested factors, two separate ANOVA were performed on DM yield data each year. Similarly, pooled ANOVAs

across years were run separately for FD level and variety factors. Due to a lack of normality, some data were square root transformed as appropriate prior to analysis. Where F-tests were significant (p<0.05), LSD was calculated to compare the means. All data were assessed for homogeneity of variance and normality and statistical analyses were performed using statistical computer software SAS (Anon., 2002).

## Results

**Overwinter survival rate comparisons:** After the first winter, number of plants were measured in early November 2008 and again in later April 2009. Almost all the varieties tested had nearly perfect overwinter survival rates while two varieties had slightly lower survival rates of 96 and 98 %, respectively. In consecutive years (2009, 2010, and 2011), all varieties appeared normal and stand persistent without visible gaps of missing plants in any plots.

**DM yield comparisons with different FD levels:** During the three production years, the temperature profile during the 3-year trial was similar to the 30-year average with the highest temperature (25-28°C) recorded in July and August and the lowest just below 0°C recorded in December and January. The average of total annual rainfalls was about 500 mm, not much different from the prior 30-year average. Generally, the rainfalls were mainly recorded between June and August during the growing seasons from year to year (Table 1). These weather variations were part of the reasons of annual total DM yield differences of the same varieties, and the interaction (year x variety) on DM yields as presented below.

Overall, the 18 varieties differed greatly in the average annual total DM yields (Table 2). In growing seasons, average forage yields of 18 varieties showed significantly in experiment ranged from 4.63 to 7.94Mg ha<sup>-1</sup>yr<sup>-1</sup>. The greatest total DM yields were produced by 'Runner' (FD2), followed by 'Goldenkey' (FD5), and 'WL-323ML' (FD4), while the smallest yields were found in 'Defi', a dormant variety (FD5), and 'CW301', another dormant variety (FD3) (Table 2).

Over a 3-year average DM yields, which differed among the three production years for all FD levels (Table 3). In 2009 year the effect of FD on Alfalfa DM yields was observed significantly (p < 0.05) because the FD2 varieties and FD4 varieties had greater DM yields (6.53 and 6.47 Mg ha<sup>-1</sup>yr<sup>-1</sup>, respectively) when compared to FD3 varieties (6.17 Mg ha<sup>-1</sup>yr<sup>-1</sup>), FD4 varieties (6.11 Mg ha<sup>-1</sup>yr<sup>-1</sup>), and FD5 varieties (6.04 Mg ha<sup>-1</sup>yr<sup>-1</sup>). while in 2010 year the effect of FD levels on alfalfa DM yields show coincident with the above results, where FD2 varieties and FD4 varieties had more DM yields (5.71 and 5.71 Mg ha<sup>-1</sup>yr<sup>-1</sup>, respectively) than FD6 varieties (6.04 Mg ha<sup>-1</sup>yr<sup>-1</sup>), with FD3 varieties and FD5 varieties being intermediate (5.51 and 5.51 Mg ha<sup>-1</sup>yr<sup>-1</sup>, respectively). In 2011, FD2 varieties and FD4 varieties had more DM yields (6.90 and 7.10 Mg ha<sup>-1</sup>yr<sup>-1</sup>, respectively) than FD3 varieties (6.60 Mg ha<sup>-1</sup>yr<sup>-1</sup>), FD5 varieties (6.63 Mg ha<sup>-1</sup> <sup>1</sup>yr<sup>-1</sup>), and FD6 varieties (6.73 Mg ha<sup>-1</sup>yr<sup>-1</sup>). Across the 3 yr, FD2 and FD4 varieties produced the greatest annual total DM yields, 5.5 and 6.1 %, respectively greater than those of FD6 varieties. Analysis of the overall data showed a very weak nonsignificant negative correlation between FD levels and annual total DM yields (r = -0.11). There was no interaction (year x FD).

 Table 3. Dry matter (DM) yields of each fall-dormancy (FD) level varieties in a field experiment conducted in North-east, China from 2008 to 2011.

ED	2009	2010	2011	Average
FD		Mg h	a <sup>-1</sup> yr <sup>-1</sup>	
2	6.53 a†	5.71a	6.90a	6.38a
3	6.17b	5.51b	6.60b	6.09b
4	6.47a	5.71a	7.10a	6.42a
5	6.11b	5.51b	6.63b	6.08b
6	6.04c	5.38c	6.73b	6.05b

 $\dagger$  Within a column, means followed by different letters are significantly different (p<0.05) according to F-protected Multiple Range Test

Overall, considering of weather condition (Table 1), there exist year effect with DM yields of > 6.09 Mg ha<sup>-1</sup>yr<sup>-1</sup> in 2009 and 2011, which are greater (p<0.05) than the other year (2010) (Table 4). Among the 3 yr, the total DM yield in 2010 was the lowest (6.09 Mg ha<sup>-1</sup> yr<sup>-1</sup>).

DM yield comparisons within the same FD levels: Among the five FD levels, ANOVA showed that varieties of the same FD level differed greatly (p < 0.05) in DM yields each year (Table 4). Within a FD level 2, the four alfalfa varieties of FD2 differed greatly (p < 0.05) in DM yields from 2009 to 2011. For example, Runner had an average annual total DM yield of 7.94 Mg ha<sup>-1</sup> yr<sup>-1</sup>, the greatest among all varieties; while 'Zhaodong', in the same FD level, produced the lowest yield (5.41 Mg ha<sup>-1</sup>  $yr^{-1}$ ). Similarly, CW201, another FD2 variety, also produced greater (p < 0.05) DM yields than that of Zhaodong in the three production years. On the contrary, varieties CW300, introduced from United States, had 15.87 % lower DM yields than Runner. Within a FD level 3, the three alfalfa varieties of FD3 differed greatly (p < 0.05) in DM yields from 2009 to 2011(Table 5). DM yields for Alfaking differed significantly by up to 30.2%

than the low yielding varieties CW301, with varieties WL-323HQ being intermediate. Within a FD level 4, the three alfalfa varieties of FD4 differed greatly (p<0.05) in DM yields from 2009 to 2011(Table 5). DM yields for Goldenkey and WL-323ML differed significantly by up to 28.5% and 28.2% respectively than the low yielding varieties WL-323, with Goldenkey and WL-323ML being nonsignificant different. Within a FD level 5, the five alfalfa varieties of FD5 differed greatly (p<0.05) in DM yields from 2009 to 2011(Table 5). DM yields for Sanditi differed significantly by up to 26.4% than the low yielding varieties Defi, with the others being nonsignificant different. Within a FD level 6, the two alfalfa varieties of FD6 were nonsignificant different in DM yields each year (Table 5).

In three production years, there was notable difference in the stability of DM yields among the same FD level varieties. For example, Runner, a stable variety, produced in all the 3 yr, great yields of 8.05 Mg ha<sup>-1</sup> in 2009, 8.12 Mg ha<sup>-1</sup> in 2010, and 8.66 Mg ha<sup>-1</sup> yr<sup>-1</sup> in 2011, respectively, whereas Goldenkey and WL-323 was an unstable variety with up to 28% differences in annual total DM yields.

Vaama	First cutting	Second cutting	Third cutting	Total yield
rears		Mg ha <sup>-1</sup> yr <sup>-1</sup>		
2009	4.33 a†	2.34 a	0.03 b	6.69 a
2010	3.81 b	2.19 b	0.09 b	6.09 b
2011	4.13 a	2.39 a	0.23 a	6.74 a

Table 4. Dry matter (DM) yields of alfalfa at each cutting averaged across all varieties in
North-east. China from 2009 to 2011.

† Within a column, means followed by different letters are significantly different (p<0.05) according to F-protected Multiple Range Test

Table 5.	Dry matter	(DM) yields o	f fall-dormancy	7 (FD)	varieties in N	North-east,	China from 20	09 to 2011.
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Variation	2009	2010	2011				
varieues	Mg ha <sup>-1</sup> yr <sup>-1</sup>						
FD2							
CW201	6.26b†	5.99b	8.35b				
Runner	8.05a	8.12a	8.66a				
WL-252HQ	6.18bc	5.91b	8.24bc				
CW300	6.09c	5.82b	8.12c				
Zhaodong	4.93d	4.71c	6.58d				
FD3							
CW301	4.52c	4.32c	6.02c				
WL-323HQ	5.91b	5.65b	7.88b				
Alfaking	6.47a	6.18a	8.63a				
FD4							
WL-323	4.99b	4.76b	6.65b				
WL-323ML	6.95a	6.64a	9.26a				
Goldenkey	6.98a	6.67a	9.31a				
FD5							
Durango	5.58a	5.33a	7.44a				
Defi	4.22c	4.03c	5.63c				
Derby	4.99bc	4.76bc	6.65b				
Sitel	5.58a	5.33a	7.44a				
Sanditi	5.73a	5.48a	7.64a				
FD6							
CW675	5.23a	5.00a	6.97a				
WL-414	5.80a	5.54a	7.73a				

† Within a column, means followed by different letters are significantly different (p<0.05) according to F-protected Multiple Range Test

## Discussion

Alfalfa is an important hay crop in the world, whose forage yield was thought to be associated with different fall dormancy levels. This finding is consistent with those of similar studies on alfalfa yield in the United States (Smith, 1961; Barnes *et al.*, 1979), and in the British Columbia(Stout and Hall, 1989). At the same time, several studies illustrated the relationship between FD and autumn forage yield, in which emphasis is given to the importance of non-FD varieties in temperate regions, where all alfalfa varieties with various FD types were able to grow. However, few studies investigated the relationship between FD and annual total DM yields and emphasized introduction of varieties are depended on annual total forage yields instead of autumn yields of the varieties. Recently, this finding is consistent with those of some Chinese studies (An *et al.*, 2003; Li and Zhu, 2005; Wang *et al.*, 2005), which showed that there appeared not to be an established relationship between FD levels and annual yields in temperate regions. Our study with 18 varieties with FD levels in northeast China with cold climate provided solid evidence to support their claim.

In fact, with cold climate in the regions of northeast China, winter survival of alfalfa crop is very important for the initial year of establishment. In our study, all alfalfa varieties with five FD levels overwintered safely in the first year (2008) with survival rates >96%, and there appeared to be no problem for any of the varieties in the subsequent years (2009 and 2010). Considering of the high winter survival rate in this study, there appeared to be associated with overall warm winter temperatures in 2009.

In this study, two key points was found. The first one was that nonsignificant correlation between annual DM yields and variety FD levels so that FD level should not be used as the main index for selecting alfalfa varieties in northeast of China. The second one was that significant differences existed in DM yields among varieties with the same FD level from our data, which implied that varieties with more DM yield potentials are much more important than FD in some regions with cold climate, which is in contrast with the current wisdom in the literature that FD level is the primary criteria for choosing alfalfa varieties in any production regions.

Through three years of yield, the magnitude of DM yield differences among most varieties tested in our research were approximate or similar, with greater DM yields in the third year (2011) than in the first year (2009), and the lowest in the second year (2010). This trend of productivity across years was similar to that reported by Nie and Yan (2005), with one exception that in their study, there was little difference in variety yields in the second production year, which was greater than that of the first year. Other researchers also noted the relationship between DM yield and production years. The main reason of DM yield differences across different experiments is associated with primary environmental conditions encountered in each study. In general, annual total yields of most varieties should be smaller in the first year than those in subsequent years as deep taproots and more lateral roots will be established as the perennial crop ages.

In summary, among the three production years, the weather conditions played an important role in this structure change in alfalfa forage production (Siddiqi et al., 2012; Li et al., 2013). Sufficient soil moisture coupled with mild air temperatures from May to June each year favored the growth of alfalfa crop for the first cuts, while excess rainfall events and the amount from June to August in the later production years led to the reduced growth rates. Likely, the general conclusions were made by Dhont et al., (2002) from North American studies where primary concerns in alfalfa production are to prepare the crop for over wintering, which were highlighted in our study for the importance of early season management to achieve annual total yields in the northeast region. In this study, annual DM yield was up to 63% in the first cut, and up to 30% of the total annual yield for the second cuts. Therefore, in the cold region, effective management practices for alfalfa production should be focused on the early period (May-July), and it is of crucial importance to increase DM yields in the first cut each year.

#### Conclusions

During three consecutive years, all varieties of five FD levels survived over the winter without any persistency problems and there were no differences in annual DM yields of varieties among FD levels 2 and 4, 3 and 5. Among the same FD varieties, DM yields for some of the dormant, semi-dormant and non-dormant varieties were found no correlated with FD levels. In conclusion, it suggests that different FD levels no effected on DM yields of alfalfa in the cold regions. At the same time, such as North-east China, effective management practices for alfalfa production should be focused on the early period (May–July), and it is of crucial importance to increase DM yields in the first cut each year.

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