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Alfalfa:
Simulated Clover Leaf Weevil Injury and Alfalfa Yield and Quality

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Abstract

The clover leaf weevil *(Hypera punctata* F.) is a sporadic but potentially serious pest of alfalfa (*Medicago sativa* L.). Feeding of newly emerged adult fourth instar clover leaf weevil was simulated in a field study at the University of Nebraska Haskell Agricultural Research Lab near Concord, Nebraska, in May 2001 and 2002. Twelve 1- by 1-m plots were arranged in a randomized complete block design with three treatments and four replications. Treatments consisted of two levels of simulated clover leaf weevil defoliation of alfalfa and an undefoliated check. Leaflets were removed over 10 d. Dry matter yield and other plant responses were measured during the first two growth cycles. Yield differences among all three treatments were significant for the first growth cycle. The relationship between percentage defoliation and percentage yield reduction was linear both years. Defoliation did not significantly affect percentages of acid detergent fiber, neutral detergent fiber, and protein. Alfalfa development also was not significantly affected in the second growth cycle. Dry matter yield reduction as a result of leaf removal at the early bud stage was the most important effect of first growth cycle defoliation.

Abbreviations: ADF, acid detergent fiber; CP, crude protein; LAI, leaf area index; NDF, neutral detergent fiber; PAR, photosynthetically active radiation.

The clover leaf weevil was introduced accidentally into the United States from Europe, with the first documented report from New York in 1881 (Tower and Fenton, 1920). Economic outbreaks of this pest tend to occur during years with mild winters followed by dry springs (Peterson et al., 1995). Although similar in appearance to the alfalfa weevil, *Hypera postica* Gyllenhal, the clover leaf weevil is larger. Unlike alfalfa weevils, clover leaf weevils overwinter as larvae and feed primarily on the lower and middle leaves of the canopy. Clover leaf weevil larvae begin pupation in mid-May. In the Midwest, adult clover leaf weevil emergence occurs from late May until mid-June (Tower and Fenton, 1920). Extensive damage has been reported on regrowth of alfalfa after the first cutting. A combination of late-stage clover leaf weevil larvae and newly emerged adults can also cause damage to first-growth alfalfa in the early bud stage (Peterson et al., 1995). In contrast, the alfalfa weevil overwinters as an adult and immediately begins feeding on alfalfa crowns in the spring. Alfalfa weevil larvae then hatch from eggs laid in stems and feed on the newly developing leaves during the first growth cycle. Both weevils injure alfalfa during the first and second growth cycle; however, the alfalfa weevil has been more thoroughly studied due to its more common occurrence.

Despite its potential importance, injury from clover leaf weevil has never been experimentally examined. Established research on early-season defoliation of alfalfa has focused on alfalfa weevil, which is a much more common pest. Both insects have been shown to be leaf tissue consumers in both the first and second growth cycle (Peterson et al., 1995). Studies involving alfalfa weevil injury include plant maturity studies by Fick and Liu (1976) and Hutchins et al. (1990). Timing and intensity of injury also has played an important part in quantifying yield loss estimates in alfalfa. Significant yield losses in the second growth cycle from alfalfa weevil injury during the first growth cycle have been reported by Berberet et al. (1981). Wilson and Quisenberry (1986) also observed second-cutting yield losses associated with larval infestations in the first growth cycle. In contrast, Godfrey and Yeargan (1987) observed no yield loss in consecutive cuttings after larval infestation during the first growth cycle. However, these studies all involved alfalfa weevil injury, not clover leaf weevil injury, on alfalfa. In particular, because clover leaf weevil defoliates lower leaves and moves up the canopy while alfalfa weevil defoliates upper leaves and moves down the canopy, differences in yield loss and regrowth responses are likely.

Insect injury on the plant can depend on the plant part injured, the type of injury, the timing of injury, and the intensity of injury (Pedigo et al., 1986). By knowing the injury parameters, yield loss estimates can be more accurately obtained.

Simulated defoliation in agroecosystems is a technique widely used to study the effect plant defoliators have on the growth and yield potential of crops. Defoliation simulates insect herbivore feeding that results in gross tissue removal. Numerous problems can occur when working with natural or artificial insect infestations, including having to place unnatural cages and using insecticides that may affect the behavior of both the insect and its host. These techniques can add unwanted factors that may produce inaccurate injury responses. Simulated techniques often provide the only means of determining damage-loss relationships. Simulated defoliation allows us to control the level of damage, its placement within the plant, and its distribution through time (Ostlie and Pedigo, 1984). Similarities in crop responses between actual and simulated defoliation must be carefully monitored to increase the fidelity of simulated defoliation experiments. A number of studies have examined the effect of simulated defoliation, including single-day defoliation (e.g., Begum and Eden, 1965; Turnipseed, 1972; Thomas et al., 1978) and sequential defoliation (e.g., Hammond and Pedigo, 1982; Higgens et al., 1984; Ostlie and Pedigo, 1984; Hunt et al., 1994) on soybean [*Glycine max* (L.) Merr.] yield and quality. Differences in plant responses may occur between single-day defoliation injury and multiple-day defoliation injury. It is essential that simulated insect injury properly mimic the spatial pattern of injury by the insect as well as the injury rate per day and the injury over multiple days (Pedigo et al., 1986).

Simulated defoliation on alfalfa has been proven to be reliable and quantifiable and has been extensively validated through research. Peterson et al. (1992a) and (1993) examined the effect of simulated alfalfa weevil injury on alfalfa. Fick (1982) showed that mechanical defoliation of whole leaflets in a greenhouse setting produced similar host responses to actual alfalfa weevil defoliation. Buntin and Pedigo (1985) found that differences between actual and simulated alfalfa defoliation were negligible. Peterson et al. (1992b) showed no significant differences in photosynthetic responses between actual and simulated alfalfa weevil injury.

The value of forage is limited by the amount of intake of digestible nutrients and by the animals' efficiency for growth (Hutchins et al., 1990). It is important to properly quantify the effect of clover leaf weevil injury on forage quality. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP) levels are often used to relate forage quality to potential livestock production. Alfalfa leaves maintain higher levels of CP than alfalfa stems (Buxton et al., 1985). Stem digestibility and CP concentrations are lowest at the lower portion of the canopy and highest at the terminal portion. Leaf digestibility remains relatively stable throughout the canopy but may exhibit a decline in CP concentration in the lower portion of the canopy (Buxton et al., 1985). Stem and leaf feed quality of alfalfa is greatly determined by the phenological development of the plant. As plants mature beyond vegetative stages, the loss of potential feed value increases (Anderson et al., 1973).

Accurate quantifications of injury and the subsequent effects on alfalfa are important. Our general objective was to examine the effect of injury on yield and forage quality of first- and second-growth alfalfa after simulated clover leaf weevil injury initiated at the early bud stage of the first growth cycle. Because clover leaf weevil feeding is a form of direct injury, effects of defoliation are expected to be associated with leaf loss. However, substantial research in soybean has demonstrated the importance of canopy light interception in mediating yield loss from defoliation (Haile et al., 1998a). Examining the possible influence of clover leaf weevil on canopy light interception and yield was another focus of this study.

Materials and Methods

This study was conducted in an alfalfa field at the University of Nebraska Haskell Agricultural Research Lab near Concord, Nebraska, in May 2001 and 2002. In 2001, the site was located in a 6.08-ha field on Pachic Haplustolls (coarse-loamy, mixed, mesic) with a 0 to 3% slope. In 2002, the site was located in a 0.69-ha field on Udic Haplustolls (fine-silty, mixed, mesic) with a 0 to 2% slope. "Garst 631" alfalfa was seeded on 1 April 2000 for the 2001 study and on 9 May 2001 for the 2002 study. The field was not irrigated during the entire season in either year, and fertilizers and herbicides were not used. Weather data was taken online from the High Plains Regional Climate Center network. The weather station used was located on site. Ten-day defoliations were initiated on 17 May 2001 and on 21 May 2002. The first-growth harvest occurred on 6 June for both 2001 and 2002. The second-growth harvest occurred on 3 July for both 2001 and 2002.

Experimental design was a randomized complete block with four replications and three treatments. The treatments consisted of an undefoliated check and two levels of simulated clover leaf weevil defoliation. Injury was imposed over a 10-d period to simulate a cohort of fourth instar and adult clover leaf weevils. Actual insect infestations were negligible during and after defoliation in both years. Management of defoliators and other pests was not needed for either year.

Defoliation treatments were based on leaf area index (LAI), which is defined as the amount of leaf area per unit ground area. Defoliation was initiated in the early bud stage to correspond to usual timing of clover leaf weevil pressure in the field. Defoliation levels were based on projected LAIs at the completion of the defoliation period. Index levels for this study represent a range of levels below alfalfa's critical LAI of 3.5 [i.e., the LAI at which 95% of photosynthetically active radiation (PAR) is intercepted by the canopy (Peterson et al., 1993)].

Final target LAIs were used to determine the treatment levels. A LAI-2000 plant canopy analyzer (LI-COR, Lincoln, NE) was used to measure LAI before and after defoliation. Photosynthetically active radiation was also measured using a 1-m line quantum sensor and data logger (Models LI-191SA and LI-1000, LI-COR, Lincoln, Nebraska) to determine light interception through the canopy before and after the experiment. The treatments consisted of a control (LAI = 4), a midlevel defoliation (LAI = 2), and a high-level defoliation (LAI = 1).

Defoliation occurred by hand-removing whole leaflets over the entire plot. Although leaves were not removed from the control plots, they did receive handling comparable to the treatment plots. Because clover leaf weevils tend to feed on the bottom and middle portions of the plant canopy, lower leaflets were removed first. Daily adjustments of projected vs. actual leaf area removed daily were made using DEFOL, a computer program written to aid in simulated insect defoliation studies (by L. Higley). Amount of leaf area removed was determined by weighing the leaflets and using subweights to run through the area meter (Model LI-3100, LI-COR, Lincoln, Nebraska) to estimate the total leaf area removed per plot. Values from the leaf area were reentered into DEFOL daily to project the subsequent leaf removal for the following day. The goal was to attain the target LAI reductions by Day 10.

Plant samples were taken after the 10-d defoliation was complete through the second cutting. Samples were taken during the first growth cycle 3 and 12 d after defoliation in 2001 and 1 and 8 d after defoliation in 2002. Plant samples were also taken 13 and 26 d after the first harvest both years. A stratified random sampling technique was used to remove 25 stems from each plot to avoid oversampling in one area, which could affect dry matter at the time of harvest. Leaf area, leaf dry weight, stem dry weights, stem lengths, nodes/

stem, and buds/stem were recorded for each plot. A 0.25-m^2 section of each plot was used to measure stem densities before each sampling period. In addition to plot yields, forage quality was also assessed. Nutrient analysis was completed at the University of Nebraska Soil and Plant Analytical Laboratory. Analyses included percentage CP, percentage ADF, and percentage NDF for all three treatments.

First-growth alfalfa harvest was initiated at 10 to 20% bloom (determined by stem sampling). Plants were harvested in two sections of the 1- by 1-m plots. A 0.5- by 0.5-m section where stems had not previously been sampled was used for harvest. A stubble residue of 7 cm was left for regrowth. The second cutting occurred 28 d after the first cutting using the same harvesting technique. Regression analyses forced through the origin were used to determine significant ($P \leq 0.05$) relationships between percentage yield reduction and percentage simulated clover leaf weevil defoliation. Regression analysis was also used to determine significant ($P \leq 0.05$) relationships among LAI, PAR, and percentage defoliation. Analysis-of-variance procedures in SAS (SAS Inst., 1990) were used, followed by least significant means separation ($P \leq 0.05$).

Regressions comparing percentage defoliation and percentage yield reduction were forced through the origin because the response was assumed to be linear. However, it is possible that a low level of defoliation may allow compensatory growth that could make it a nonlinear relationship. For this experiment, we assumed the response to be linear since defoliation injury had an immediate impact on dry matter yield. The 2-wk period between defoliation and harvest may not have been adequate for compensatory growth to be a factor.

Results and Discussion

Rainfall amounts and average daily temperature during the 10-d defoliation period were 28.4 mm and 14.8°C in 2001 and 3.6 mm and 15.6°C in 2002. The total rainfall and average daily temperature 1 to 40 d after defoliation were 67.8 mm and 19.9°C in 2001 and 73.1 mm and 22.1°C 1 to 44 d after defoliation in 2002. Due to adequate rainfall during the study, the alfalfa was not water stressed in 2001 or 2002. Percentage defoliation, LAIs, and canopy light interception by treatment were similar for both years after the defoliation period (table 1).

Table 1. Summary of percentage defoliation, percentage light interception of the canopy, and leaf area index (LAI) by treatment (± SE) of alfalfa at the completion of the simulated clover leaf weevil defoliation period

Target LAI	2001	2002
	Simulated clover leaf weevil defoliation	
	%	
Check	0	0
2.0	60.7 ± 1.71	45.1 ± 1.30
1.0	73.4 ± 1.36	68.1 ± 0.27
	Light interception	
	%	
Check	95.6 ± 1.13	92.1 ± 0.65
2.0	81.7 ± 1.24	82.9 ± 1.18
1.0	65.9 ± 1.52	65.9 ± 0.87
	LAI	
Check	4.7 ± 0.18	4.1 ± 0.05
2.0	1.8 ± 0.08	2.3 ± 0.05
1.0	1.2 ± 0.06	1.3 ± 0.01

First-Growth Yield Responses

Stem densities were not significantly different between treatments at any time during the first and second growth cycle for both 2001 and 2002 ($P > 0.05$). These data suggest that clover leaf weevil injury does not simulate lateral branching in alfalfa and are supported by similar findings of alfalfa weevil injury (Peterson et al., 1993).

The most significant effect of simulated clover leaf weevil feeding at the early bud stage was the loss of alfalfa leaf tissue. Leaf dry weights differed among treatments for both sampling dates of the first growth cycle in 2001 and 2002 (table 2). Stems were least affected by defoliation injury as indicated by stem length and weight data taken during the first growth sampling (data not shown). A reduction in stem lengths occurred 8 d after defoliation and was significant at the 0.10 level. Stem lengths were not significantly different 3 and 12 d after defoliation in 2001 and 1 d after defoliation in 2002. With the exception of the second sampling date on Day 8 in 2002 ($F = 9.27$; df = 2,9; $P = 0.0065$), stem dry weights were not significantly lower for both sampling dates of the first growth cycle for both 2001 and 2002 ($P > 0.05$) (table 2).

Table 2. Leaf and stem dry weight means (± SE) by treatment at two dates postdefoliation during the first alfalfa growth cycle

Percentage defoliation by treatment	Leaf dry weight		Percentage defoliation by treatment	Stem dry weight	
%	g		%	g	
			2001		
	3 d postdefoliation	12 d postdefoliation		3 d postdefoliation	12 d postdefoliation
0	0.331 ± 0.019a*	0.367 ± 0.016a	0	0.541 ± 0.049a	0.635 ± 0.023a
60.7 ± 1.71	0.206 ± 0.017b	0.204 ± 0.023b	60.7 ± 1.71	0.544 ± 0.048a	0.488 ± 0.045b
73.4 ± 1.36	0.120 ± 0.018c	0.181 ± 0.015b	73.4 ± 1.36	0.463 ± 0.050a	0.515 ± 0.047ab
$F_{2,9}$	33.78	30.38	$F_{2,9}$	0.89	3.86
$P > F$	0.0001	0.0001	$P > F$	0.4455	0.0616
			2002		
	1 d postdefoliation	8 d postdefoliation		1 d postdefoliation	8 d postdefoliation
0	0.326 ± 0.020a	0.353 ± 0.029a	0	0.451 ± 0.046a	0.519 ± 0.027a
45.1 ± 1.30	0.207 ± 0.008b	0.244 ± 0.022b	45.1 ± 1.30	0.370 ± 0.020a	0.413 ± 0.032ab
68.1 ± 0.27	0.144 ± 0.006c	0.176 ± 0.015c	68.1 ± 0.27	0.343 ± 0.028a	0.343 ± 0.028b
$F_{2,9}$	54.03	15.43	$F_{2,9}$	2.90	9.27
$P > F$	0.0001	0.0001	$P > F$	0.1066	0.0065

* Means followed by the same letter within a column are not significantly different ($P \geq 0.05$; LSmeans test).

First-growth alfalfa yield differed among treatments in both years (table 3). The relationship between percentage simulated defoliation by clover leaf weevil and percentage reduction in dry matter yield was linear in both 2001 ($n = 12$; $\beta \pm$ SE, 0.505 ± 0.030; $r^2 = 0.96$; $P \leq 0.01$) and 2002 ($n = 12$; $\beta \pm$ SE, 0.466 ± 0.056; $r^2 = 0.89$; $P \leq 0.01$) (fig. 1). A negative relationship between PAR intercepted by the canopy and percentage dry matter yield reduction was also linear in both 2001 ($n = 12$; $\beta \pm$ SE, -1.200 ± 0.185; $r^2 = 0.81$; $P \leq 0.01$) and 2002 ($n = 12$; $\beta \pm$ SE, -1.33 ± 0.081; $r^2 = 0.96$; $P \leq 0.01$) (fig. 2). There was also a negative linear relationship between LAI and percentage dry matter yield reductions in 2001 ($n = 12$; $\beta \pm$ SE, -10.67 ± 0.765; $r^2 = 0.95$; $P \leq 0.01$) and 2002 ($n = 12$; $\beta \pm$ SE, -12.10 ± 1.365; $r^2 = 0.89$; $P \leq 0.01$). Slopes were not significantly different both years for each variable regressed on percentage yield reduction in dry matter yields, and quadratic relationships between parameters were not significant in either year. Leaves in the upper portion of the plant canopy are more photosynthetically active. Since clover leaf weevil injury occurs on the lower portion of the alfalfa canopy, LAI and PAR would not be as affected. In this study, a combined slope of 5.82 kg ha^{-1} yield loss per milligram leaf dry weight removed per stem was recorded for clover leaf weevil injury. In contrast, Peterson et al. (1993) showed a dry matter yield loss relationship of 7.70 kg ha^{-1} yield loss per milligram leaf dry weight removed per stem for alfalfa weevil injury, indicating a more significant impact on yield loss.

Table 3. Dry matter yields (± SE) of first and second growth cycles of alfalfa by treatment and year

Defoliation	First cutting	Second cutting
%	g m⁻²	
	2001	
0	509.4 ± 30.03a*	193.2 ± 15.16a
60.7 ± 1.71	356.5 ± 12.26b	203.1 ± 12.90a
73.4 ± 1.36	317.2 ± 2.38b	193.3 ± 13.26a
$F_{2,9}$	29.25	0.17
$P > F$	0.0001	0.8466
	2002	
0	403.6 ± 19.75a	146.8 ± 5.35a
45.1 ± 1.30	342.1 ± 7.94b	150.3 ± 6.48a
68.1 ± 0.27	260.3 ± 10.82c	153.9 ± 6.50a
$F_{2,9}$	27.16	0.33
$P > F$	0.0002	0.7240

* Means followed by the same letter within a column are not statistically different ($P \geq 0.05$; LSmeans test).

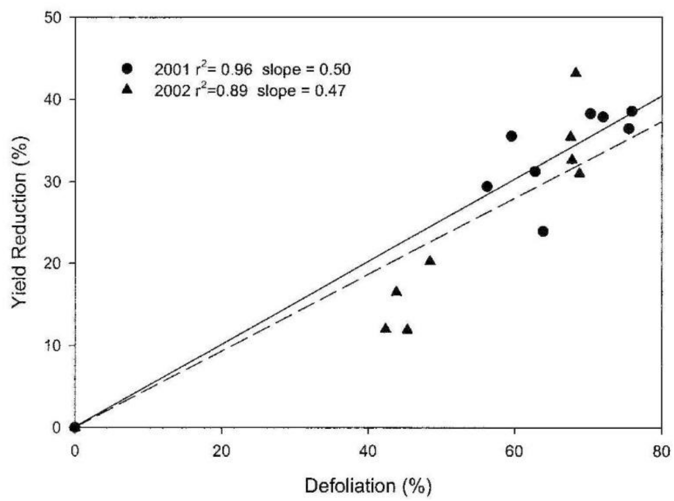

Figure 1. Regression (forced through origin) of percentage simulated clover leaf weevil defoliation on percentage alfalfa dry matter yield reduction for 2001 and 2002; solid line, 2001; dashed line, 2002.

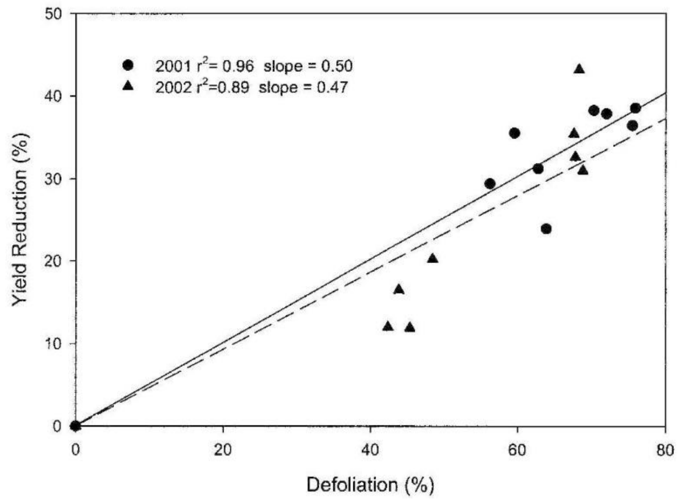

Figure 2. Regression of percentage canopy light interception on percentage yield reduction for 2001 and 2002; solid line, 2001; dashed line, 2002.

Forage Quality Analysis

Forage quality analysis of first-cutting samples showed no significant differences between treatments in percentage ADF [2001 (F = 1.41; df = 2,9; P = 0.2942); 2002 (F = 1.90; df = 2,9; P = 0.2048)], percentage NDF [2001 (F = 0.25; df = 2,9; P = 0.7865); 2002 (F = 3.01; df = 2,9; P = 0.0997)], and percentage CP [2001 (F = 0.27; df = 2,9; P = 0.7686); 2002 (F = 0.79; df = 2,9; P = 0.4831)] after the defoliation period. Berberet and McNew (1986) found that damage from alfalfa weevil may reduce the forage quality of alfalfa. Top feeding of the alfalfa weevil can impact forage quality more than the removal of the lower leaves, which are not as photosynthetically active and thus are of less forage value. Unlike crown leaves, leaves on the lower portion of the plant canopy naturally senesce or are sometimes lost by disease such as black stem (*Phoma medicaginis* var. *medicaginis).* Therefore, tissue lost to the clover leaf weevil also may be subject to other tissue loss factors. These factors may not be as significant with the alfalfa weevil, as it feeds on the upper portion of the plant canopy.

Second-Growth Yield Responses

During the 2 yr of sampling during the second growth cycle, only one sample period impacted leaf or stem dry weights. Leaf dry weights were greater 13-d postharvest in 2002 from treatments that had been defoliated during the previous growth period (F = 8.41; df = 2,9; P = 0.0087). Data at other sampling periods did show a trend toward this result as well. Increased sunlight penetration through the canopy after insect defoliation may encourage earlier regrowth. Although significant at 13-d post first harvest, there was no difference among treatments in forage dry matter yield in the second harvest.

These results are similar to results indicated by Peterson et al. (1993) for the alfalfa weevil. Both sets of data suggest gross tissue removal throughout the canopy has a negligible effect on second-growth harvest of alfalfa.

Conclusions

Results indicate the effect of clover leaf weevil injury differs from that of the alfalfa weevil, beyond consumption rate. From a quantitative perspective, yield effects change depending on which portion of the canopy is being defoliated. Although both weevils reduce dry matter yield, injury from clover leaf weevil is not as substantial. Removal of lower leaves does not impact LAI and PAR as significantly. Top leaves are more photosynthetically active and may be more important for dry matter accumulation. From a qualitative perspective, effects on forage quality from clover leaf weevil injury as it relates to percentage ADF, percentage NDF, and percentage CP were negligible, suggesting that removal of leaves from the lower portion of the canopy has little effect on quality of the hay. Lower leaves have more indigestible parts and may not impact forage quality as much as leaves removed from the upper portion of the canopy. Similarities between the two weevils also exist. In this study, differences in regrowth were negligible, especially during the second growth cycle. Leaf tissue removed does not initiate additional stem growth. A linear relationship was shown between canopy light interception and yield loss. The relationship between yield loss and percentage defoliation is linear, suggesting that yield loss from clover leaf weevil feeding is a result of leaf tissue removal. Although similarities such as these exist between the two weevils, the differences in consumption rate and location of injury can be used to develop economic injury levels specifically for clover leaf weevil injury on alfalfa.

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