MODELING POLLINATION FACTORS THAT INFLUENCE ALFALFA SEED YIELD IN NORTH-CENTRAL NEVADA

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ABSTRACT

The relative importance of both environmental and management factors on alfalfa seed yield was investigated on North–Central Nevada farms. Multiple linear regression models using 2002-2003 data revealed that cumulative tripped flowers increased seed yield in both years. Field location does not appear to make a difference in the observed variation in tripped flower production. The results suggest that seed yield can be increased by (a) by placing bee shelters closer and (b) cultural practices that increase total flower production. Both these factors increased tripped flowers and thus had a positive effect on yield. In addition, warmer temperatures during the growing season, particularly in the early stages of plant growth is shown to not only increase tripped flowers but also reduce the time when the maximum tripped flowers occur. The latter appears to have a significant influence on cumulative tripped flowers and thus the total seed yield.

Key words; alfalfa seed, pollination, leafcutting bees

INTRODUCTION

Alfalfa (Medicago sativa L) seed production has traditionally been concentrated in the western United States [4]. At the time of the last census, California, Idaho, and Washington were the top three states with total area of almost 22257 ha of production. However, Kansas, Montana, Nevada, Oregon, and South Dakota acreages totaled 13013 ha. Other states with alfalfa seed production also included Wyoming, Utah, Arizona, Oklahoma, Nebraska, and New York.

Entomologists and agronomists have studied a wide range of alfalfa seed production issues, especially those related to pollinators such as the alfalfa leafcutting bee (Megachile rotundata). Alfalfa seed production in the western United States relies exclusively on irrigation. Because alfalfa seed producers were relatively late in adopting commercial pollination practices to their management practices, there are still many unanswered questions concerning leafcutter bee management [6]. Previously, the alkali bee (Nomia melanderi) was also used for pollination but the leafcutting bee has to a large extent replaced it as the main pollinator for alfalfa seed in the western United States [1; 9].

Bee production problems require continued study because it has become increasingly difficult for producers to over-winter their bees [5]. In fact many producers now automatically purchase additional bees every season. Richards, K.W [8] documented the life cycle and natural history of leafcutters. In their article "Current Status of the Alfalfa Leafcutting Bee as a Pollinator of Alfalfa Seed" Petersen [7] addressed a wide range of issues, among them economic parameters on different population levels since the cost of bees can represent as much as 25 percent of the total production costs. Flower production and pollination models have been developed in order to try and understand the relationship between bee population, rate of bloom, and the timing within the season of maximum pollination [12]. These studies have contributed to greater knowledge about alfalfa seed production and the many interrelated factors that ultimately affect yield [11].

Flower tripping rates and bee species were examined [3] to see if there was any difference in pod production and seed set. The role of temperature regimes on the survival, emergence, and longevity of leafcutting bees has also been studied [5]. Their work involving leafcutter population dynamics, foraging behavior, and alfalfa bloom, pollination rates and seed yield among alfalfa seed fields in eastern Oregon suggested that similar yields could be achieved with smaller bee populations if bee release dates were better timed to correspond to alfalfa bloom [2].

Cumulative tripped flower count is the sum of the weekly tripped flower count over the season. Thus increasing the weekly tripped flower count would increase the cumulative tripped flowers and this would increase the seed yield. Growers are faced with the task of coordinating the agronomic conditions of their alfalfa fields (i.e. soil, plant water relationships) and the production and release of bees at the correct time to try and maximize their weekly tripped flowers in order to increase yields. Growers must carefully time the nesting of their bees to correspond with the bloom of alfalfa. Normally nesting occurs around the middle of June in central Nevada. However, weather variables such as temperature can directly affect bee movement and thus have an effect on pollination and hence the tripped flower production.

The results to date suggest the relationship between seed yield and flower tripping is complex. As a general rule, the more overall flowers produced and then tripped, the larger the yield. However, the total number of flowers produced and tripped can be influenced by weather, alfalfa variety, irrigation and soils, and the proximity to bee population and timing of bee nesting. Stephenson, A.G [10] showed that in most plants, including alfalfa, that if "fully pollinated' only one-half of fertilized flowers produce fruit. The earlier the plants are pollinated and set seed, the more likely they are to avoid the adverse effects of inclement weather having an impact on the yield. In addition, it is also important to know, upon the release of bees, how the tripped flower count changes weekly (time profile of weekly tripped flower production) over the growing season. Specifically, it is important to know the shape of the time profile since this may have an influence on seed yield.

Weekly tripped flower production typically increases in the early part of the growing season and starts to progressively decrease in later weeks with the weekly tripped flower count reaching the maximum sometime between the week after the bee release and the time of seed harvest (Figure 1). However, the question is what time profile (reaching maximum tripped flower production early versus late) results in higher cumulative tripped flowers at the end of the season. The curve marked A in figure 1a shows the maximum of weekly tripped flower count occurring early in the season. The curve marked B in figure 1a shows the maximum of weekly tripped flower count occurring later in the season. The curves A1 and B1 in figure 1b show the reverse situation with B1 resulting in higher cumulative tripped flowers than A1. It seems reasonable that reaching the weekly tripped flower count maximum during the early season results in higher cumulative tripped flower count than a delay in reaching the maximum weekly tripped flower count

Figure 1A

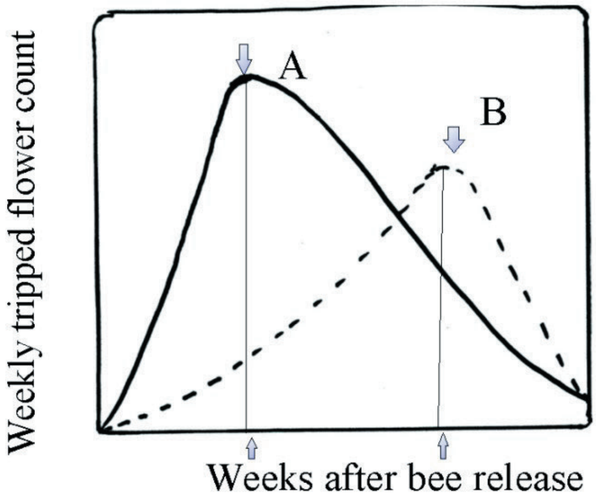


Figure 1B

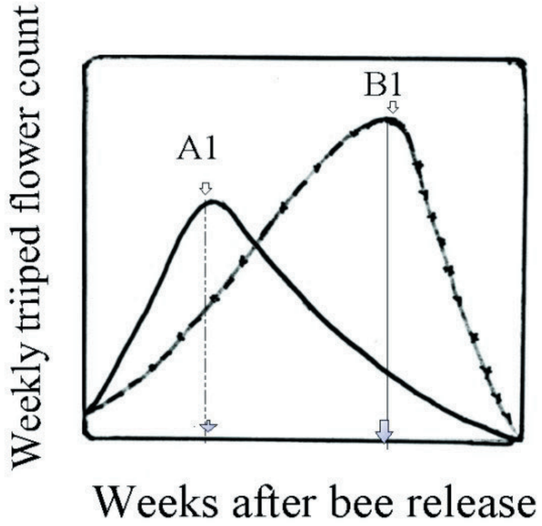


Figure 1 Possible time profiles of weekly tripped flower counts

due to the possibility of achieving early pollination and set seed. Distance between the bee domiciles is also an important criterion. As a general rule, growers try to place at least one bee domicile for every 3 to 4 ha. Four to five gallons of bees (1 gallon = approximately 10,000 bees) are utilized for each acre. If the weather doesn't cooperate during the initial flush of bloom, all is not lost.

The key questions that need to be addressed are: Whether and to what extent cumulative tripped flower count over the season affects alfalfa seed yield in North-Central Nevada? How does the time profile of weekly tripped flower counts (reaching maximum tripped flower production early versus late) influence the cumulative tripped flower count? How does alfalfa seed yield respond to the week when the maximum weekly tripped flower count is reached? How and to what extent do factors such as total weekly bloom, proximity to the bee box, temperature and time (week) after the bee release affect the weekly tripped flower production?

MATERIALS AND METHODS

There are two principal areas of alfalfa seed production in Nevada. These areas are both in the north-central part of the state; Lovelock and Orovada. This study took place on-farm in Lovelock, Nevada. It was a cooperative effort between University of Nevada Cooperative Extension, University of Nevada College of Agriculture, Biotechnology, and Natural Resources, Nevada Department of Agriculture, Nevada Seed Council, local producers, industry representatives, and the USDA/ARS Bee Lab at Logan, Utah.

During the 2002 growing season, a total of six farms (L1, L2, B1, B2, and N1 and N2) and in 2003, four farms (L1, L2, N1 and N2) participated in the study. During the growing seasons, each farm had two fields. Each field consisted of one alfalfa leafcutting bee shelter and 15 tagged plants. Normal production management techniques require that shelters be spaced within a field at the rate of approximately one shelter per 4 ha. Alfalfa leafcutting bees were stocked at a rate of 11 to 12.5 gallons per ha; each gallon containing approximately 10,000 bees. In the Lovelock area, bees are normally released around early to mid June depending on weather conditions. It is imperative that the bee nesting be timed with the onset of flower production so that the seed harvest concludes before any fall rains (Bosch and Kemp, 2005).

At each of the plots five plants were tagged 10 meters in front of a shelter, five more at 40 meters, and another five at 80 meters. At each tagged plant, the racemes on the east half (surveyor facing north) were banded at their base, examined and the number of tripped and un-tripped flowers were recorded. Each plot was then visited on a regular weekly interval starting in mid-June at the onset of flower production and bee release and continuing through mid-August when few if any flowers are being produced. At this late time in the growing season, even if flowers are pollinated they will not develop into viable seed by the scheduled harvest date. Daily high and low temperatures were also recorded. At each weekly visit the tripped and untripped flowers were again recorded. At harvest time all fruits from the east half of the fifteen

Table 1 Descriptive statistics of the response and the predictor variables used in the multiple linear regression models.

Variable	Mean	Minimum	Maximum
Y (Weekly tripped flower count)	4.80	0.00	46.60
Log Y (Log of weekly tripped flower count)	0.79	-2.30	3.84
TF (Weekly total flower count)	40.27	0.00	293.67
LTF (Log of Weekly total flower count)	2.80	-2.30	5.68
LMT (Log of mean weekly air temperature 0^{C})	1.358	1.214	1.452
week2 (Week-squared)	36.28	1.00	100.00
week (Week)	5.44	1.00	10.00
DIST (distance from the bee box) in m	43.33	10.00	80.00
LDIST (Log distance from the bee box)	3.46	2.30	4.38
WKLMT (Week x LMT)	23.47	4.12	43.00
LMTTF (LMT x TF)	12.02	-10.14	23.48
Seed Yield (2002) kg	871.68	490.90	1622.37
Seed Yield (2003) kg	790.85	558.31	1015.20
Total tripped flower count	41.2	21	92
X1 Log of Cumulative total flower count at the week of maximum tripped flower.	4.6	3.5	6.4
X2 Week of maximum tripped flower occurred	4.2	2	8

Table 2. The multiple linear regression model parameters and their significance levels between log of weekly tripped flower count and many predictors variables described in model(2).

Model terms	Parameter estimate	*t-statistic and P-value*	
β_0 *Intercept (field N2 and year 2002))*	-64.59288	-7.45	<.0001
β_2 *Year*	-0.28468	-2.54	0.0117
β_1 *Field: L1*	0.18725	1.28	0.2015
β_1 *Field: L2*	0.01002	0.07	0.9449
β_1 *Field: B1*	-0.13637	-0.72	0.4725
β_1 *Field: B2*	0.30823	1.64	0.1014
β_1 *Field: N1*	-0.16611	-1.15	0.2521
β_3 *Log (total flower) LTF*	0.55550	14.90	<.0001
β_4 *Week WK*	14.30502	9.85	<.0001
β_5 Wk^2	-0.02583	-2.51	0.0126
β_6 *Log (mean weekly temperature) LMT*	14.97856	7.20	<.0001
β_8 *WK*LMT*	-3.27697	-9.43	<.0001
β_7 *Log(distance)*	-0.10458	-2.01	0.0451
R^2	0.7797		
R^2 *adjusted*	0.7689		

plants at each plot surveyed were collected. The number of pods and seeds per pod were recorded for each of the fifteen plants at each of the plots.

Modeling procedure: The following general linear model (1) is used to examine whether and what extent total cumulative tripped flowers account for alfalfa seed yield.

$$Y_1 = \beta_0 + \beta_1 X_i + \beta_2 Year + \acute{\varepsilon} \qquad (1)$$

Where Y_1 = Alfalfa seed Yield (in kg) in the i^{th} farm in j^{th} year

β_0 = Y intercept for year 2002

β_1 = Partial Regression coefficient – Change in the alfalfa seed yield (kg) associated with a unit change in cumulative tripped flower count

X_i = Mean Cumulative tripped flower count in the i^{th} field in j^{th} year.

β_2 = Differences in seed yield between year 2003 and

Table 3 the estimates of the week of maximum tripped flower production and its 95% confidence intervals estimated by the non-linear parameter estimates using the SAS/STAT NLMIXED procedure.

Field / year	*Estimated Week ± SE when Maximum tripped flower*	*t Value*	*95% Lower*	*95% Upper*
L1 in year 2002	4.92 ±0.64	7.67	3.6644	6.1942
L2 in year 2002	4.92±0.64	7.67	3.6644	6.1942
N1 in year 2002	6.10 ±0.50	12.21	5.1243	7.0952
N2 in year 2002	6.10 ±0.50	12.21	5.1243	7.0952
B1 in year 2002	6.10 ±0.50	12.21	5.1243	7.0952
B2 in year 2002	6.10 ±0.50	12.21	5.1243	7.0952
L1 in year 2003	5.76 ±0.50	11.46	4.7759	6.7576
L2 in year 2003	5.76 ±0.50	11.46	4.7759	6.7576
N1 in year 2003	5.76 ±0.50	11.46	4.7759	6.7576
N2 in year 2003	5.76 ±0.50	11.46	4.7759	6.7576

Table 4: Multiple linear regression model estimates (model 3) predicting the log of cumulative tripped flowers at the end of the season.

Model terms	*DF*	*Parameter Estimate*	*Standard Error*	*t Value*	*Pr > |t|*
β_0: Intercept	1	0.53911	0.48908	1.10	0.2783
β_1: Year (0=2002; 1:2003)	1	-0.33250	0.09584	-3.47	0.0015
β_2: log of cumulative total flower count at end of season (X1)	1	0.65520	0.07956	8.24	<.0001
β_3: Week at maximum tripped occurred (X2)	1	-0.15867	0.03832	-4.14	0.0002
R^2: 0.7508 Adj. R^2: 0.7281 N =36					

2002

Year $_=$ (0 for year 2002; 1 for year 2003)

έ = Random error – normally distributed with 0 mean and equal variance.

A general linear model (2) is developed to analyze the effects of both management and environmental factors such as the distance from the bee shelter, mean weekly air temperature during the flower production, weekly total flower production, and the number of weeks after the bee release on the weekly tripped flower production. A log transformed response and continuous predictor variables were fitted in the model to satisfy the statistical assumptions of the general linear model.

$$Y_2 = \beta_0 + \beta_1 Field_i + \beta_2 Year + \beta_3 LTF + \beta_4 WK + \beta_5 WK^2 + \beta_6 LMT + \beta_7 Ldist + \beta_8 WK*LMT + έ \quad (2)$$

Where $Y_{2} =$ Log of weekly tripped flower count in the i^{th} field in j^{th} year

β_0= Y intercept for year 2002 and field N2

β_{1i}= Differences in weekly tripped flower count between field N2 and other fields.

$Field_{i} =$ (i= 1 to 6 fields and field N2 is the reference site)

β_2= Differences in weekly tripped flower count between year 2003 and 2002

LTF $_{and}$ β_3 = Log of weekly total flower count and its partial regression coefficient

WK $_{and}$ β_4 = Number of weeks after the release of bees and its linear regression coefficient

WK^2 $_{and}$ β_5 = Square of the number of weeks after the release of bees and its quadratic regression coefficient

LMT $_{and}$ β_6 = Log of mean weekly Air temperature and its partial regression coefficient

Ldist $_{and}$ β_7 = Log of distance from the bee box and its partial regression coefficient

β_8 $_=$ Interaction coefficients between WK*LMT .

$\acute{\varepsilon}_{ij}$ = Random error – normally distributed with 0 mean and equal variance.

A general linear model (3) is used to test the research hypothesis whether the cumulative tripped flower production influenced by the week of maximum tripped flower count occurred, i.e. whether higher alfalfa seed yield is obtained by advancing the week of maximum tripped flower count. In other words, how does alfalfa seed yield respond to the week when the maximum weekly tripped flower count is reached?

$$Y_3 = \beta_0 + \beta_1 Year + \beta_2 X_1 + \beta_3 X_2 + \acute{\varepsilon} \qquad \text{-- (3)}$$

Where Y_3 = Log of cumulative tripped flower count in the in year (2002 and 2003)

β_0= Y intercept for year 2002

β_1= Differences in seed yield between year 2003 and 2002

Year= Year (0 for year 2002; 1 for year 2003)

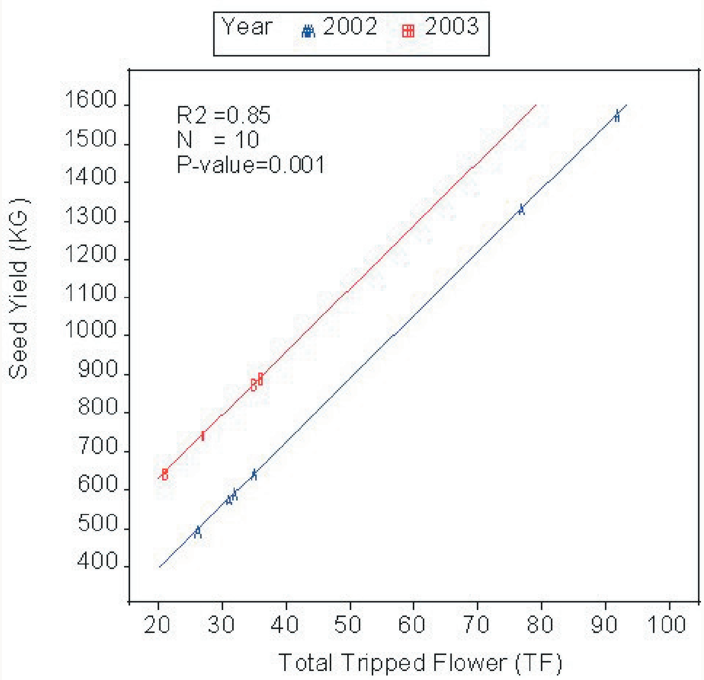
β_2= Partial Regression coefficient for year – Change in the log of cumulative tripped flower count associated with a unit change in log of cumulative total flower count

X_1 = Log of Cumulative total flower count.

β_3= Partial Regression coefficient – Change in the log of cumulative tripped flower count associated with a unit change in week of maximum tripped flower count occurred.

X_2 = week of maximum tripped flower count occurred.

$\acute{\varepsilon}$ = Random error – normally distributed with 0 mean and equal variance.



Parameter		Estimate	Standard Error	t Value	Pr > |t|
Intercept		302.7688903	115.5506935	2.62	0.0344
YEAR	2002	-232.2475028	120.4548828	-1.93	0.0952
YEAR	2003	0.0000000	.	.	.
Total Tripped Flower		16.4059485	2.6401962	6.21	0.0004

Figure 2 Regression model (1) between mean alfalfa seed yield(kg) and the total tripped flower count / plot in two years

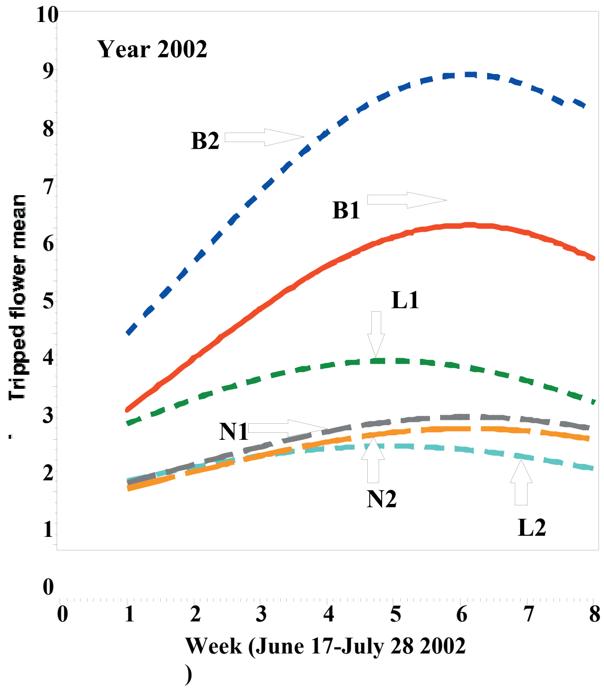


Figure 3 Predicted tripped alfalfa flower means at the mean temperature level in different fields in year 2002

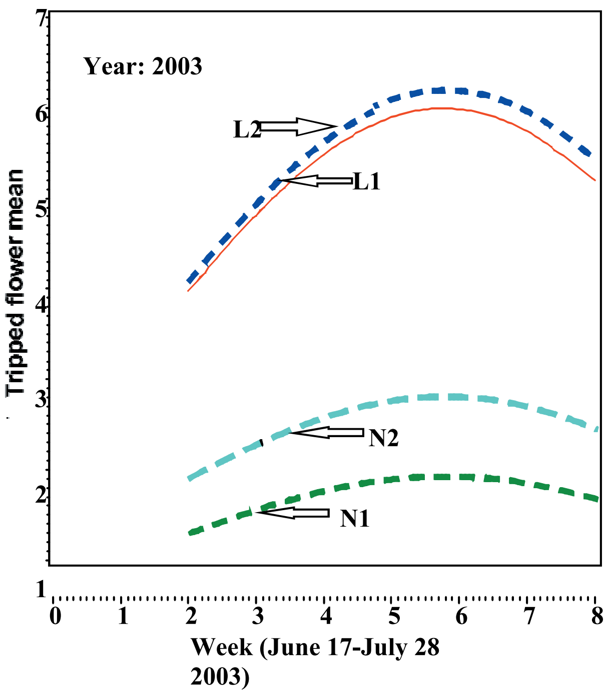


Figure 4 Predicted tripped alfalfa flower means at the mean temperature level in different fields in year 2003

All statistical models used in this study were performed using the SAS software version 9.13. The SAS/STAT REG procedure was used to fit all three regression models (1-3). The SAS/STAT NLMIXED procedure was used to estimate and test the non-linear parameter, the week of maximum tripped flower production and its 95% confidence intervals for a given field and a given year estimated by the non-linear parameter estimates from the general linear regression model (2).

RESULTS

The fitted regression model (1) between mean alfalfa seed yield and the total tripped flower count /plot in two years is presented in Figure 2. The overall regression model was statistically significant (P-value 0.0014, N=10 R^2=0.85). Correlation between the cumulative tripped flower production and the alfalfa seed yield in both years is positive and statistically significant (t-value 6.21) thus, leading to rejection of the null hypothesis that cumulative tripped flower production has no influence on yield.

The fitted regression model parameters and their significance levels (2) between log of mean weekly tripped flower count and all other predictor variables described in model 2 are presented in Table2. The overall full and the reduced regression model were statistically significant (P-value < 0.001, N=200 R^2= 0.78). The log distance from the bee box to alfalfa plant is negatively correlated (P-value < 0.05) with the mean weekly tripped flower count. Overall the log of weekly tripped flower is positively correlated with the weekly mean temperature (P-value < 0.0001). The log of tripped flowers showed significant relationship with the interaction term involving log of weekly total flower count and log of weekly air temperature. The weekly tripped flower count shows a quadratic trend with the time (weeks) from the time of bee release (P-value < 0.01). The weekly tripped flower count reached a maximum tripped flower count between 4.9 weeks to 6.1 weeks in all fields in both years (Table 2 and Figure 3-4).

The results of multiple linear regression models 3 clearly showed a significant negative correlation (P-value < 0.001) between cumulative tripped flower count at the end of the season and the time of maximum tripped flower production (Table 3). Thus, higher alfalfa seed yield is expected when the maximum tripped flower count occurs early.

Discussion

Results of the regression equation specified by model 1 indicate that if cumulative tripped flowers can be increased, the seed yield will also increase. In fact, it can be shown that a 10 percent increase in cumulative tripped flowers will approximately increase seed yield by 8% at the mean levels. The percent increase in seed yield will be lower when tripped flowers are less than the mean and higher when they are greater than the mean. Yield differences between the two years of approximately 227 kg are noticeable and are due to factors not included in the model.

From the estimated second general linear model, it is clear that there is a significant negative relationship between tripped flowers and distance from the bee box. A 10 percent decrease in the distance from the bee box translates to about 1 percent increase in tripped flower count. If one wants to increase yield by increasing the tripped flowers, it will be necessary to place the bee shelters closer. Thus, from an economic view point, the cost of more bee shelters will have to be weighed against gains from potential increase in tripped flowers and thus the seed yield.

The relationship between the mean air temperature (MTF) has a positive relationship to tripped flowers in the initial growing season. However, as a result of the interaction term WK*LMT, the effect of MTF on tripped flowers becomes zero at around 4 ½ weeks and then the effect becomes negative.

The weekly count of tripped flowers increases at a diminishing rate early in the season, reaches a maximum and starts declining later in the season as indicated by the significant positive coefficient associated with WK and the smaller but significant negative coefficient associated with WK^2. The maximum seems to occur between 4.9 and 6.1 weeks at the mean values of other variables. The point at which the maximum tripped flower occurs is again related to the mean temperature. The higher the mean temperature, the earlier the maximum tripped flowers occur.

Tripped flowers are positively related to the total flowers. The total flower count is assumed to be exogenous in this study and is often a function of cultural practices influenced by such decisions as the level and timing of irrigation, soil fertility, etc. However, from the results of this analysis, a 10 percent increase in total flowers will approximately lead to 5.5 percent increase in tripped flowers. Therefore, the economic cost of measures to increase total flower production would have to be weighted against the economic gains resulting from increased tripped flowers and thus the yield.

The regression results indicate that the coefficients associated with the sites are not significantly different from zero. In other words, the field's locations do not appear to make a difference in explaining the tripped flowers and there is no comparative advantage to any field. However, there appears to be an extremely small but statistically significant difference among the two years in the intercept estimate.

Finally, from the third general model, the cumulative tripped flower over the season is clearly impacted by not only the cumulative total flower count but also when the maximum of the tripped flowers occur. If the cumulative total flower count increases by 10 percent, it would lead to a 65 percent increase in cumulative tripped flowers. If the week in which the maximum tripped flowers occur can be reduced by one week, the tripped flower count would go up by about 15 percent.

SUMMARY

The analysis suggests management strategies to increase the seed yield a) by placing bee shelters closer and b) cultural practices that increase total flower production. Both these factors will increase tripped flowers and thus will have a positive effect on yield. In addition, warmer temperatures during the growing season, particularly in the early stages of plant growth is shown to not only increase tripped flowers but also reduce the time when the maximum tripped flowers occur. The latter appears to have a significant influence on cumulative tripped flowers and thus the total seed yield.

This study provides some key suggestions for alfalfa seed producers. It is certainly possible to move the bee shelters closer together. However, producers have no control over temperatures. At the present time, producers already vary the incubation period in order to time their bee release with warmer temperatures. Changing other cultural practices to increase overall flower production is somewhat problematic. The USDA Bee Biology Laboratory at Utah State University and other scientists continue to conduct studies on bee pollination dynamics, fertility requirements, and more efficient and productive irrigation regimes to assist producers in increasing their yields.

While the analysis of this study suggests that higher early tripped flower counts will result in greater yields, the authors fully recognize that it is a complicated process involving a symbiotic relationship among cultural and agronomic practices. Additional research, especially in the area of bee pollination dynamics, will be required to provide additional answers to these complicated questions.

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