The effects of misting upon seed yield of birdsfoot trefoil, Lotus corniculatus L., and the relationship of moisture content of pod, seed and fruit at dehiscence
by Mark Andrew Hughes

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Agronomy
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Abstract:
Seed pod dehiscence is the major factor limiting birdsfoot trefoil seed production. Birdsfoot trefoil has an indeterminate growth habit resulting in all stages of pod maturation occurring on the same plant at the same time. As a result many mature pods dehisce before being harvested. Research has shown that although 784-1008 kg/ha of seed is possible, only 155-224 kg/ha can usually be harvested with conventional harvest methods because of seed pod dehiscence.

Investigations into increasing seed yields of birdsfoot trefoil through use of an irrigation management program (misting) were conducted at the Field Research Laboratory, Bozeman, MT in 1979 and 1980. Misting treatments were: 1) no misting, 2) twice daily misting, and 3) hourly misting. Seed yields were obtained on four harvest dates in both years. In addition, laboratory experiments were conducted to determine the moisture percentage of pods at dehiscence, and evaluate the variation of moisture at dehiscence of four clones of birdsfoot trefoil.

Pod dehiscence occurred at approximately 10% moisture. In 1979, misting delayed pod dehiscence and allowed immature pods to mature prior to harvesting for seed. Maximum seed yield and seed viability were obtained with twice daily misting and harvesting when approximately 70% of all pods were brown. Hourly misting decreased pod dehiscence and increased yield, but resulted in lower seed viability due to moldy seed and seed pods, and seeds germinating in the pod.

In 1980, cool, wet conditions were encountered during seed pod maturation. These conditions delayed pod maturation. Misting was not necessary, and when used (for purposes of this experiment) decreased seed yield.

These data suggest that in dry areas of the western United States, it may be possible to prevent pod dehiscence through periodic misting of the birdsfoot trefoil canopy with sprinkler irrigation.

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ABSTRACT

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LITERATURE REVIEW

Birdsfoot trefoil (*Lotus corniculatus* L.), is native to the British Isles and the region from the Mediterranean Sea northward to the Scandinavian Peninsula (15, 24, 37). McKee and Schoth (24) reported that it was indigenous to Europe, except in Lapland and Northern Russia, and is largely an alpine plant in Southern Europe. They reported its occurrence also in Africa, Asia, and Australia and its absence in America. MacDonald (21) reported that the species is not native of the Western Hemisphere, but was introduced from Europe as an impurity in imported seed.

Birdsfoot trefoil is a long-lived perennial, non-bloating, and generally nondemanding in its moisture, fertility and grazing requirements (12, 37). It is an important forage crop in certain areas of the United States suitable for growing alfalfa, red clover, or white clover (21, 30). No other irrigated pasture legume equals birdsfoot trefoil in its duration and ability to withstand continuous grazing (40).

Factors limiting the successful use of this crop are relatively low forage yields (43), small seed size (10, 11), lack of seedling vigor (13, 43), seed pod dehiscence (3, 22, 25, 32), high seed cost and inadequate supplies of seed (3, 21, 23, 25, 32, 36).

Morphological Characteristics

Birdsfoot trefoil has 20-70 ovules per ovary, averaging about 45. Usually, only an average of about 20 ovules per ovary develop into mature seed (8, 36). Budar (8) found that ovules within an individual ovary vary considerably in rate of development. Because of this, ovaries contain some fertilizable ovules for 8-10 days, although individual ovules are fertilizable for only 2 or 3 days (36).

After pollination, pods develop rapidly reaching maximum length in about 3 weeks. The color of the pods changes from dark green or purple to light green, tan, light brown and finally to brown or black. Seeds become phyisiologically mature slightly before or at the time pods turn light brown (3, 23, 36). Wiggans, et al. (46) noted that seeds mature 7-10 days before pod dehiscence. Rate of pod development is influenced by weather conditions. S. R. Anderson (3), working in Iowa, found that mature pods and seeds formed 24-47 days after pollination, while in New York, Winch (47) found that the same stage of development required 26-38 days.

Seed set in birdsfoot trefoil depends on pollination of flowers by insects. This is accomplished primarily by various species of pollen and nectar collecting honey bees (Hymenoptera), which are capable of tripping the flowers (5). Morse (27) found that honey bee populations of one bee per 0.9 square meter or 2.5 colonies (hives) per hectare are needed for maximum seed production.

Cultural Practices

The prostrate growth habit of birdsfoot trefoil reduces seed yields (21). Anderson and Metcalfe (4) noted reduced lodging and increased seed yields when birdsfoot trefoil was grown with Kentucky bluegrass, orchardgrass, or timothy. Highest seed yields were obtained in the birdsfoot trefoil-Kentucky bluegrass mixture. In contrast other researchers (23, 36) have indicated that pure stands of birdsfoot trefoil produce the highest seed yields. They indicate that as the amount of grass increased, the seed yield declined.

Clipping in the spring delays flowering and seed set, and extends the period of seed harvest (36). Delaying seed harvesting may reduce seed pod dehiscence by coinciding with cooler weather or higher humidity environments. Winch (47) reported that clipping birdsfoot trefoil at early bud stage delayed seed harvest seven days. Other studies (4, 5) indicated that spring and early summer clipping reduces seed yields in comparison to unclipped stands.

All cultivars of *Lotus corniculatus* L. flower and set seed over an extended period of time; usually four to six weeks (21, 36, 37). Individual plants will have flowers, ripe pods, and some dehisced pods (3, 23, 24, 29, 32, 47). This indeterminate flowering and seed set makes harvest timing critical for maximum seed yield. Harvesting too early reduces yield and results in immature and nonviable seed. Harvesting too late reduces yield due to pod dehiscence (3, 32, 36, 40).

Several methods have been used to harvest seed (23). The most common method used in the Northeast is to mow or windrow, and then combine. The windrow is allowed to dry before combining. This method can result in excessive seed losses during the dry down period of the forage. Direct combining of birdsfoot trefoil has been used but, in general, is not acceptable. Large quantities of green forage go through the combine, which slows harvest and causes seed loss by clogging of the harvester (40). Chemical desiccants and defoliants successfully defoliate and dry the plants before harvest which aids in direct combining. Jones (18) reported that University of California scientists found that dinitro herbicides aided in harvesting of birdsfoot trefoil seed. He states:

> . . .when dinitro compounds are properly used as a defoliant, birdsfoot trefoil can be combined without significant loss of seed through shattering. Proper irrigation practices will eliminate much of the seed loss that usually occurs prior to harvest.

Cooper (14) was first to report the use of sodium cyanamid as a deterrent to pod shattering. He concluded that 27 kg/ha of sodium cyanamid applied just prior to harvest resulted in complete foliage kill of birdsfoot trefoil and delayed pod dehiscence. However, spraying too early results in seed pod dehiscence whether the pods are fully mature or not.

Desiccants and defoliants are not extensively used in the harvest of birdsfoot trefoil seed at the present time. Wiggins, et al. (46)

experimenting with desiccant sprays concluded that "the natural variability of climatic conditions, especially temperature and relative humidity, will affect seed pod dehiscence, regardless of the harvest method." Wiesner, et al. (45) found that the application of abscissic acid, benzyladenine, and gibberellic acid did not affect seed or forage yield of 'Leo' birdsfoot trefoil.

Some new seed harvesting machinery has been developed for handling legume crops which have a tendency to lodge. Vance (42) used a machine which had a sickle that cut the forage at the soil surface. This machine had a strong vacuum, which functions just over and ahead of the sickle and pulls up the foliage, loose seed heads, and shattered seed. Vance (42) estimates that this machine saved 98 percent of the seed. Wiesner, et al. (44) is investigating the use of a vacuum harvester to pick up dehisced seed from the soil surface. He has been able to pick up birdsfoot trefoil seed from the soil surface. However, separating the seed from trash has been a problem.

Harvesting techniques and irrigation intervals influence hard seed content of birdsfoot trefoil (1, 20). One-hundred percent of birdsfoot trefoil seed may be hard when fully ripe; however, the seed coat is usually scarified by the threshing action of the combine. Seed viability and germination depends upon its maturity (time of harvest), harvest method, cleaning, processing, and storage. Abu-Shakre, et al. (1) reported that hard seed content of alfalfa was influenced by irrigation interval. Hard seed content increased with decreased irrigation frequency.

Improvement of Seed Yield and Prevention of Pod Dehiscence

The lack of an efficient means for harvesting birdsfoot trefoil seed has prompted extensive breeding to increase seed yield and prevent pod dehiscence. Components of seed yield and associated characteristics, (2, 9), morphological and physiological plant characteristics (17, 26) have been studied.

MacDonald (21) was the first to study the relationship of plant characteristics in birdsfoot trefoil to seed development and seed yield. Since then, many workers have reported similar studies in many different species.

Similarities in agronomic characteristics influencing seed yield are found among legume species. Taylor, et al. (39) reported that the number of heads per plant in red clover was the primary factor governing seed yield. Number of seeds per head and seed weight were of less importance. High yielding progenies were earlier than average to flower. Hawkins (16), in England, reported that factors affecting seed yield of red clover appear to be number of seeds set per head, seed size, number of heads, and resistance to disease. Seed set, seed size, and number of heads are so closely linked that differences in yield are indicated by differences in any one of these characters (16).

Pedersen and Nye (31) measured seed yield components in alfalfa for three varieties. 'Uinta' had 8.0 pods per raceme, 'Ranger' had 7.4, and 'Lohontan' had 6.7. 'Uinta' had 4.1 seeds per pod, 'Ranger' had 4.0, and 'Lahontan' had 3.6. These results correspond to actual seed yields with 'Uinta' being the highest and 'Lahontan' the lowest. In this study, the number of flowers per raceme in alfalfa was not significantly associated with seed yield.

Albrechtsen, et al. (2) and Buzzell and Wilsie (9) reported that the number of umbels setting seed in birdsfoot trefoil had the greatest influence upon seed yield. Studies in the North Central Region (26) indicated that the phenotypic characteristics correlated with seed yields were pods per umbel, and seeds per umbel. These researchers suggest that no single independent variable accounts for the variation in seed yields among clones grown in different locations. Bresciani (6) indicates that number of umbels per plant and number of pods per umbel were significantly correlated to seed yield. Number of seeds per pod was also significantly correlated with seed yield. Albrechtsen, et al. (2) states, "seed yield is the end result of the interrelationships of many component factors and the environment."

Acknowledgment of the relationships among characters that affect forage and seed yield is necessary before selection for improvement of both can be made.

Peacock and Wilsie (30) selected for vegetative vigor and seed setting in clones of birdsfoot trefoil. They did not find an increase in seed production in either of the first or second recurrent selection; however, there were individual second cycle crosses which were superior in seed production. They suggested that crossing of selected superior second cycle parents should give increases in seed set.

In a similar study using 'Leo' birdsfoot trefoil and recurrent selection, Sandha, et al. (34) were able to increase seed yield with two cycles of selection. The geno-phenotypic method was superior to the genotypic method. They indicated that further improvement with additional cycles of selection should be possible with the geno-phenotypic method. Sandha, et al. (35) also used a poly-cross progeny testing method and found a significant association between seed yield and seed size, seeds per pod, and pods per inflorescence. The positive correlation between seed yield and seed size does not agree with earlier reports by Twamley (41) and Albrechtsen, et al. (2).

Genetic variation exists for seed yield (9). Hereditability estimates suggest that a large part of phenotypic variance for seed set is genetically controlled (19). Although selections for high seed yields have been incorporated into experimental synthetics, no varieties have been developed specifically for higher seed yields (36).

Based on these breeding studies to increase seed yield, it appears that the genetic seed yield potential of birdsfoot trefoil will not be realized unless an efficient method for preventing pod dehiscence is found. In addition, Wiesner, et al. (45) reports that the pods containing large seed shatter first because they were the first to mature, leaving only the small immature seeds on the plant for harvesting. Therefore, any significant improvement in seed size will be reduced due to the seed dehiscent problem of birdsfoot trefoil.

Selection for resistance to seed pod dehiscence in birdsfoot trefoil was first attempted by Peacock and Wilsie (29). They found wide differences in susceptibility due to pod dehiscence among clones. Seed pod dehiscence was reduced 17 percent with one selection cycle for shatter resistance. An interspecific-hybridization study was undertaken by Phillips and Keim (33), in an attempt to incorporate the indehiscent seed pod character of L. coimbrensis into the agronomically desirable L. corniculatus. They noted that pod dehiscence was directly associated with relative humidity. Crosses of L. corniculatus with the L. coimbrensis produce no seeds due to high flower drop and low pollen fertility.

Birdsfoot trefoil pods are tough and do not shatter when the relative humidity is above 35 percent (25, 32, 36, 40). Peterson, et al. (32) noted that irrigation is necessary to maintain a canopy of new growth above most of the seed pods, which keeps the humidity high enough to reduce seed pod dehiscence. Metcalfe, et al. (25) have done the majority of the work with pod dehiscence, relative humidity, and moisture equilibrium. The incidence of pod dehiscence is greatest when temperatures are high and the relative humidity is low. They studied the effects of variations in relative humidity on pod dehiscence and determined the moisture equilibrium between mature pods and the surrounding atmosphere. Field studies were also made to measure temperature fluctuations both within the pod and on its surface under direct and shaded sunlight. They found: 1) birdsfoot trefoil pods dehisce at approximately 30 percent relative humidity but not at 35 percent, and 2) the moisture equilibrium value of the pod at 30 percent relative humidity was 10.05 percent, and at 40 percent relative humidity, the moisture equilibrium was 10.49 percent. These values suggest a very close interdependence of relative humidity, moisture equilibrium, and pod shatter. These field studies indicated temperatures within and at the surface of mature pods varied as much as 12 degrees C. from the air temperature depending on cloud cover.

The structural pattern of tissues in birdsfoot trefoil pods is related to the mechanism of dehiscence. Buckovic (7) studied the anatomical structure of birdsfoot trefoil pods and found that the pod wall was composed of two separate layers. He concluded that moisture loss was the governing factor in pod dehiscence and postulated that the rate of moisture loss differed in the two tissues. This resulted in tensions between individual layers of fibers and possibly in their component fibers. As the dry down continued the tension overcomes the cohesion at the sutures and the two halves of the pod separate and twist open. Pods readily dehisced when they had lost between 38 to 60 percent of their original moisture, but the moisture content of pods at dehiscence was not determined. Pod moisture at shatter needs to be determined in order to aid farmers in harvesting of birdsfoot trefoil seed.

These studies suggest that seed production would be most successful in geographical areas where relative humidity would be above 40 percent. In addition, Winch and MacDonald (48) recommended that harvesting should begin when 70-80 percent of the pods are mature, i.e., when light brown to brown. However, Anderson (3) suggested slightly earlier harvest, "when maximum number of pods are light green to light brown."

CHAPTER I

THE EFFECTS OF MISTING ON PHYSIOLOGICAL DEVELOPMENT, POD DEHISCENCE, AND SEED YIELD OF BIRDSFOOT TREFOIL (*LOTUS CORNICULATUS* L.)

Introduction

Birdsfoot trefoil (*Lotus corniculatus* L.) is a valuable forage legume (12, 37). Its use has been limited due to poor stand establishment and high seed cost. The high seed cost is due to low seed yields which result from seed pod dehiscence. Pod dehiscence is less of a problem in humid than in arid regions. However, seed production potential is greater in arid regions under irrigation due to the lower incidence of disease (23, 32).

The lack of an efficient means for harvesting birdsfoot trefoil seed has prompted extensive breeding to increase seed set, yield, and prevent pod dehiscence (2, 9, 30). Plant breeders have been unable to develop indehiscent cultivars of birdsfoot trefoil (34, 36); thus improvement in seed yield must come from better management.

Metcalfe, et al. (25) found that birdsfoot trefoil pods dehisce readily at 30 percent relative humidity but not at 40 percent. Moisture equilibrium of pods at these relative humidity levels was 10.05 percent and 10.39 percent, respectively. They reported a close relationship between relative humidity and moisture content at time of pod dehiscence.

In the dry areas of the western United States, it may be possible to prevent pod dehiscence through periodic wetting of the birdsfoot trefoil canopy with sprinkler irrigation. The objective of this study was to determine the effects of various misting levels on pod dehiscence and seed yield of birdsfoot trefoil.

Materials and Methods

A one-ha seed field of 'Tretana' birdsfoot trefoil was planted in 1978 at the Field Research Laboratory, Bozeman, MT, into a Bozeman silt loam (Argic-Pachic Cryoborall) soil. Seeding rate was 2.25 kg/ha pure live seed with rows spaced 0.6 m apart. The effects of three misting treatments and four harvest dates on seed yield of birdsfoot trefoil were studied using a split-plot-randomized-complete-block design with three replications. Main plots were: 1) no misting, 2) twice daily misting (10AM and 3PM), and 3) hourly misting (8AM to 6PM). The four harvest dates were considered the sub-plots. In 1979, seed pods matured rapidly due to an unusually hot, dry summer and seed was harvested at 7-day intervals starting on July 27. In 1980, seed pods matured slowly due to an abnormally cool, wet summer which resulted in harvest dates of August 25, September 4, September 15, and October 2.

Plots were 9.14 m^2 with a 6.1 m border between plots within replications. Replications were separated by a minimum of a 15.2 m border. These borders insured against irrigation overlap during windy conditions.

Sencor [4-amino-6-tert-butyl-3-(methylthio)-as-triazin-5(4H)-one] was applied at .68 kg/ha active ingredient in the spring of 1979 and 1980 to control weeds.

The irrigation system consisted of 9.14 m sections of polyvinylchloride (PVC) pipe joined by PVC couplers and elbows. Model P-J25 Rainbird sprinkler heads with 0.397 cm nozzle opening were attached to 1.9 x 92.0 cm galvanized risers located in the corner of each plot. Each sprinkler head misted a quarter circle with the spray distance (9.14 m) controlled by an adjustable aluminum flap. Plots were watered for 3 minutes at 3.45×10^5 Newtons m^{-2} (50 psi) pressure. Time of application for individual plots was controlled by gate valves. Plots were misted daily (except when it rained) from July 27 to August 17 in 1979, and from August 25 to October 2 in 1980.

At each harvest date, 1 m^2 areas were randomly chosen within each misting treatment and 15 randomly selected stems were collected from within the area for detailed morphological characterization. The remaining vegetation was then harvested at ground level, sacked, air-dried for 30 days, and the seed cleaned with a belt thrasher (without air), sieves, and an Oregon continuous blower. Clean seed was weighed and kg/ha yield of seed calculated.

Data collected from the 15 randomly selected stems, from each plot, included the number of buds/stem, flowering umbels/stem (no pods), fertilized umbels/stem (umbels with pods), pod color, non-shattered pods/stem, shattered pods/stem (and their position on each stem), total number of pods/stem, total wet fruit weight/stem, total air dried fruit weight/ stem, and fruit moisture percentage (air dry basis) at harvest (1979 only).

Pod color was determined using a Munsell (28) color chart for vegetative plant tissue. Pods were classified into four categories: dark green-purple (Hue 2.5R3/6 - Hue 5GY6/8), light green (Hue 2.5 GY8/6), tan (Hue 5Y8/4), and brown (Hue 5YR4/6). At each harvest date the total number of pods in each color category was recorded. Percent pods in each category was determined by dividing the number of pods in that category by the total number of pods (including shattered pods) and multiplying by 100.

Position of shattered pods was recorded by dividing the stem into quarters. Shattered pods in the top quarter were assigned a value of one and shattered pods below the top quarter were given a value of two.

Moisture percentage at harvest was determined by subtracting air dry fruit weight, after 30 days of drying, from wet fruit weight, dividing by air dry fruit weight, and multiplying by 100.

Seed quality was determined in the laboratory for each misting-harvest date combination treatment. Four groups of 100 seeds from each treatment and harvest date were surface sterilized (0.1% NaOCl or Tetrachloro-parabenzoquinone, 98 percent), placed in plastic germination boxes on moist blotter paper, and germinated in a dark germinator at

20 C. Seeds were watered as needed. After 12 days counts were taken on the number of germinated, abnormal, hard, and dead seeds. A completely random design with four replications was used. Replications were placed in the germinators on consecutive days. One hundred seed weight for each treatment and harvest date was determined.

Data were analyzed using analysis of variance for a split-plot randomized complete block design and means were separated with Duncan's New Multiple Range Test (38).

Results and Discussion

Environmental conditions in 1979 were ideal for birdsfoot trefoil seed production (Table 1). The dry, hot conditions resulted in an excellent environment for high seed production and pod dehiscence.

Misting significantly changed flower and seed maturation characteristics including number of buds, number of brown pods, total number of pods, number of shattered pods, pod moisture, and seed yield (Table 2). Misting did not affect number of flowering and fertilized umbels, and the number of dark green, light green and tan pods (Table 2). Significant differences among harvest dates were detected for all traits, except number of flowering and fertilized umbels and number of tan pods (Table 3).

Misting caused increased bud production at the later stages of seed maturation (Tables 2 and 3). However, nonsignificant differences among misting treatments and harvest dates for number of flowering and

Table 1. Weather summary for 1979-80 at Bozeman, MT.

		Mean		
		Maximum Temperature	Minimum Temperature	Accumulation Precipitation
30-yr Avg.	May	17.39	3.61	65.25
1979	May	17.78	3.89	58.25
1980	May	18.94	5.28	142.00
30-yr Avg.	June	21.22	7.17	81.75
1979	June	23.50	8.44	91.50
1980	June	20.11	7.83	71.25
30-yr Avg.	July	27.50	10.61	29.75
1979	July	28.50	11.39	14.75
1980	July	27.50	11.28	28.00
30-yr Avg.	August	26.83	9.67	29.75
1979	August	27.06	11.17	37.75
1980	August	24.78	9.00	46.25
30-yr Avg.	September	20.83	5.28	44.00
1979	September	26.61	7.33	1.75
1980	September	21.22	6.50	85.00
30-yr Avg.	October	14.83	7.78	1152.50
1979	October	17.22	2.33	38.50
1980	October	15.44	1.06	18.25

Table 2. The effects of three misting treatments upon seed yield components of birdsfoot trefoil at Bozeman, MT, 1979.

Morphological Characteristics	Misting Treatments		
	None	Twice Daily	Hourly
Buds (No.)	0.08 c[a]	1.08 b	1.92 a
Flower Umbels (No.)	0.00	0.08	0.25
Fertilized Umbels (No.)	5.40	6.40	5.60
Dark Green Pods (No.)	2.70	7.90	6.50
Light Green Pods (No.)	2.60	2.80	2.90
Tan Pods (No.)	1.20	1.30	1.40
Brown Pods (No.)	3.40 b	8.30 a	8.20 a
Total Pods (No.)	10.10 b	20.20 a	17.00 a
Shattered Pods (No.)	5.60 a	0.90 b	0.80 b
Pod Moisture (%)	33.60 b	51.00 a	58.30 a
Seed Yield (kg/ha)	294.00 b	554.00 a	551.00 a

[a]Means in the same row followed by the same letter are not significantly different by Duncan's multiple Range Test at p = .05 level.

Table 3. The effects of four harvest dates upon seed yield components of birdsfoot trefoil at Bozeman, MT, 1979.

Morphological Characteristics	Harvest Dates			
	July 27	August 3	August 10	August 17
Buds (No.)	0.67 b[a]	0.11 b	1.11 ab	2.22 a
Flowering Umbels (No.)	0.33	0.11	0.11	0.00
Fertilized Umbels (No.)	5.30	6.30	6.00	5.50
Dark Green Pods (No.)	9.50 a	9.70 a	2.10 b	1.40 b
Light Green Pods (No.)	4.70 a	3.90 ab	1.60 ab	0.80 b
Tan Pods (No.)	1.00	1.70	1.30	1.10
Brown Pods (No.)	0.80 b	3.10 b	10.70 a	9.30 a
Total Pods (No.)	15.90 ab	18.40 a	15.60 ab	13.00 b
Shattered Pods (No.)	0.00 c	1.60 bc	3.00 b	5.20 a
Pod Moisture (%)	68.20 a	60.10 a	28.10 b	34.10 b
Seed Yield (kg/ha)	320.00 c	510.00 a	567.00 a	467.00 ac

[a]Means in the same row followed by the same letter are not significantly different by Duncan's Multiple Range Test at p = .05 level.

fertilized umbels (Tables 2 and 3) indicate flower drop probably occurred and these increased buds did not result in increased seed yields. Evidently flowers were not pollinated or the environment was not conducive to seed development of newly formed pods during the misting period.

The greatest number of dark green pods were detected early in the season and declined with maturation (Table 3). This decrease was due to maturation of green pods and failure of new pods to develop. A similar relationship was detected for light green pods, but of a lesser magnitude (Table 3).

Misting treatments greatly increased the percentage of brown pods with increasing maturity (Figure 1). This was due to decreased shattering. Misting twice daily reduced pod dehiscence and increased seed yield. Additional wetting delayed pod maturation and decreased yields. Hourly misting of birdsfoot trefoil resulted in a maximum of 55 percent brown pods (Figure 1), whereas misting twice daily resulted in 70 percent brown pods (Figure 1). Seventy percent brown pods were obtained on the same harvest date (August 10) as the maximum seed yield was produced. The 70 percent brown pod stage could be used by seed producers to determine time of harvest, if a misting program was being used. This value would rarely be obtained without misting under warm, dry conditions because pod shatter would occur first. The percentage of brown pods was significantly correlated to total seed yield ($r = 0.45^{**}$).

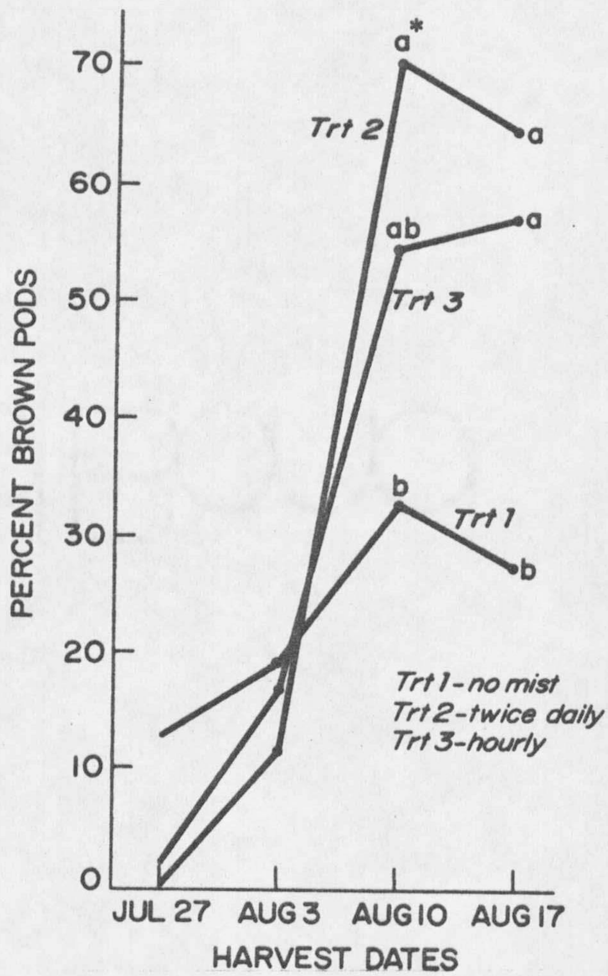

Figure 1. The effects of three misting treatments and four harvest dates upon percent brown pods of birdsfoot trefoil at Bozeman, MT, 1979.

*Means in the same row followed by the same letter are not significantly different by Duncan's Multiple Range Test at p = .05 level.

Twice-daily and hourly misting increased total number of pods (Table 2), and this factor was significantly correlated with seed yield at all harvest dates except the harvest on July 27. The correlations for harvest dates of August 3, 10, and 17 were: r = 0.67**, 0.81**, and 0.76**, respectively. The increased number of brown pods, total number of pods, and seed yield resulted from a decrease in pod dehiscence due to misting (Figure 2). Pod dehiscence increased with maturity for all treatments and was highest for the non-misted treatment (70 percent). Pod dehiscence for the misting treatments did not exceed 12 percent. The number of shattered pods was negatively correlated (r = -0.72**) with seed yield on August 17.

Misting increased pod moisture (Figure 3) and prevented pod dehiscence. Pod moisture was significantly correlated with seed yield on August 10 and 17 (0.75** and 0.67**, respectively).

Misting twice daily increased seed weight (Table 4) due to the retention of heavier seed. In contrast, heavier seed shattered in the non-misted birdsfoot trefoil treatments and could not be harvested (44).

Misting increased seed yield by preventing pod dehiscence and increasing seed weight of harvested seed (Figure 4). The seed yield obtained on August 10 for the misting treatments is indicative of maximizing seed production while minimizing pod dehiscence. No significant differences were detected between the twice daily and hourly mistings.

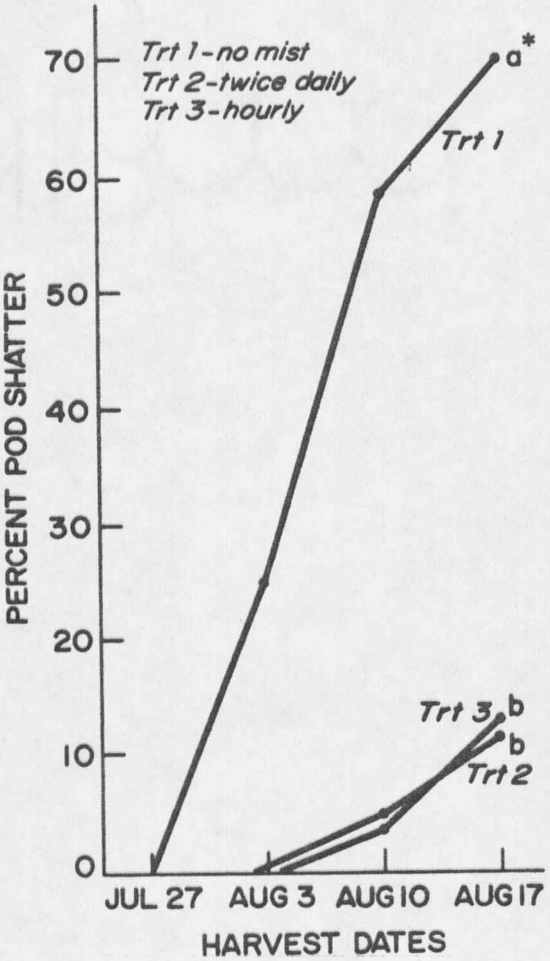

Figure 2. The effects of three misting treatments and four harvest dates upon percent shattered pods of birdsfoot trefoil at Bozeman, MT, 1979.

*Means in the same row followed by the same letter are not significantly different by Duncan's Multiple Range Test at p = .05 level.

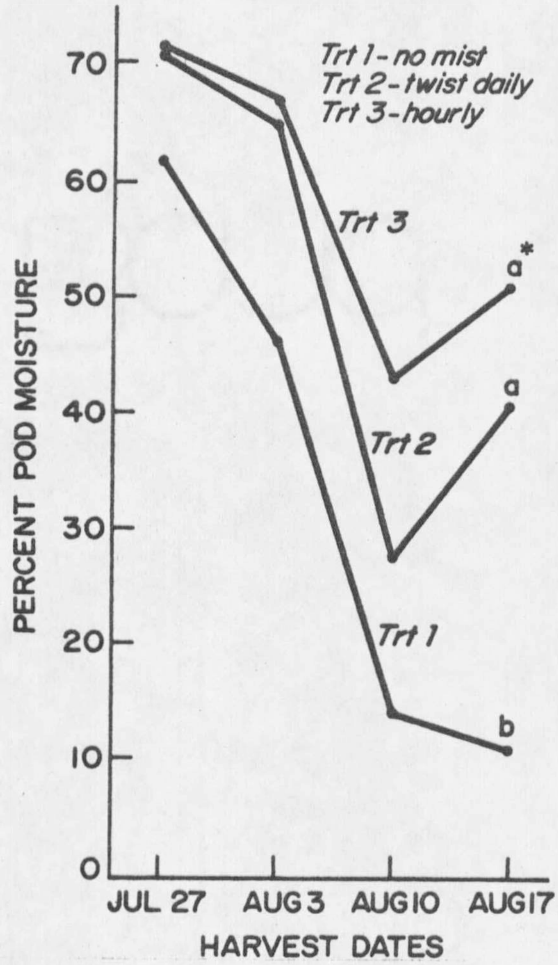

Figure 3. The effects of three misting treatments and four harvest dates upon percent pod moisture of birdsfoot trefoil at Bozeman, MT, 1979.

*Means in the same row followed by the same letter are not significantly different by Duncan's Multiple Range Test at p = .05 level.

Table 4. The effects of three misting treatments upon total seed weight per 100 seed at four harvest dates of birdsfoot trefoil at Bozeman, MT, 1979.

Misting Treatments	July 27	August 3	August 10	August 17
None	1.90	1.78	1.22 b*	0.82 b
Twice daily	1.49	0.19	5.98 a	4.21 a
Hourly	1.77	2.93	4.46 ab	3.80 ab

*Means in the same row followed by the same letter are not significantly different by Duncan's Multiple Range Test at p = .05 level.

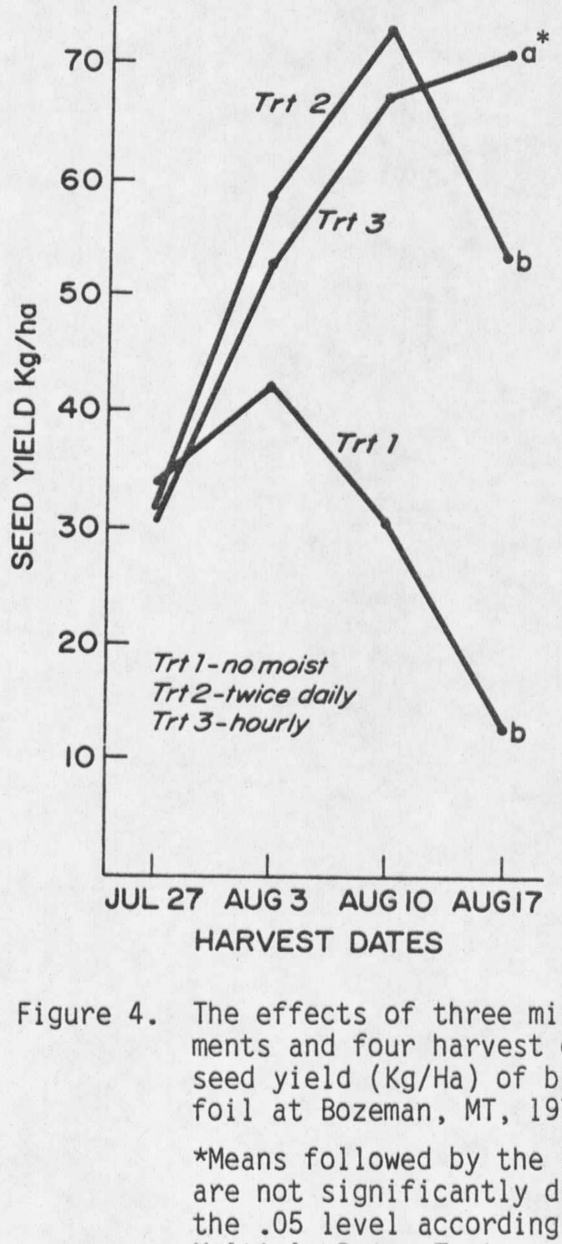

Figure 4. The effects of three misting treatments and four harvest dates upon seed yield (Kg/Ha) of birdsfoot trefoil at Bozeman, MT, 1979.

*Means followed by the same letter are not significantly different at the .05 level according to Duncan's Multiple Range Test.

twice daily misting was unable to prevent brown pods from shattering between August 10 and 17.

Seed viability was not affected by misting twice daily (Table 5); but the hourly misting decreased viability due to excessive moisture which caused seed germination in the field. The hourly misting also caused fungal growth within and on seed pods. Seed viability increased with later harvests (Table 6).

Sencor may have repressed the vegetative growth and maturation of birdsfoot trefoil in 1980. Maturation was further delayed by cool, wet conditions in August and September of 1980 (Table 1). The first harvest in 1980 was August 25 as compared to July 27 in 1979. The combination of Sencor damage and environment seemingly reduced seed yields in 1980 (Figure 5). The effects of misting treatments on flowering and seed maturation characteristics were generally non-significant. Seed yields in 1980 were decreased due to misting treatments, indicating that misting should not have occurred as it further delayed pod maturation.

In 1979 pod moisture was related to pod dehiscence and was increased by misting. Light misting prevented mature pods from dehiscing while immature pods developed. Maximum seed yields were obtained by misting twice daily and harvesting when 70 percent of the pods are brown (August 10, 1979). The twice daily misting of birdsfoot trefoil had significantly better seed germination than did hourly misting, indicating that excessive moisture results in poor seed viability. Therefore, seed

Table 5. The effects of three misting treatments upon percent viability of seed lots of birdsfoot trefoil at Bozeman, MT, 1979.

Treatment	Percent Viability
1	80.97 a
2	73.23 a
3	54.43 b

*Means followed by the same letter are not significantly different at the .05 level according to Duncan's Multiple Range Test.

Table 6. The effects of four harvest dates on percent viability of seed lots of birdsfoot trefoil at Bozeman, MT, 1979.

Harvest Dates	Percent Viability
July 27	54.35 b
August 3	69.95 a
August 10	76.84 a
August 17	77.05 a

*Means followed by the same letter are not significantly different at the .05 level according to Duncan's Multiple Range Test.

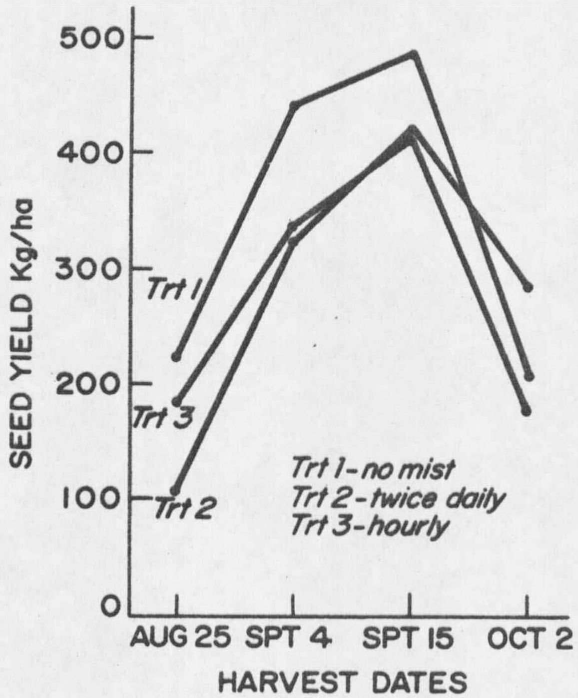

Figure 5. The effects of three misting treatments and four harvest dates upon seed yield (Kg/Ha) of birdsfoot trefoil at Bozeman, MT, 1980.

producers should apply only enough water to prevent pod dehiscence. It is possible that daily misting would have been sufficient.

Although misting of the birdsfoot trefoil canopy twice daily maximized seed yields, seed producers must base the irrigation schedule on the environmental conditions present. When environmental conditions are such that pod dehiscence will not occur, misting of pods will not increase seed yield.

When producing birdsfoot trefoil seed in an area which has low relative humidity and misting is used, I suggest that 1) start misting when brown pod moisture declines to approximately 10 percent as indicated in Chapter II, 2) mist twice daily or as needed to prevent shatter, 3) swath when the environmental conditions dictate or at approximately 70 percent brown pod stage, and 4) combine as soon as forage will pass through combine. If environmental conditions are such that wet cool conditions might prevail late in the season, I advise combining prior to the 70 percent brown pod stage. We have observed that frequent wetting of the birdsfoot trefoil canopy produces a leathery, tough pod which does not shatter as readily as non-misted pods.

CHAPTER II

THE RELATIONSHIP OF MOISTURE PERCENTAGE OF POD, SEED, AND FRUIT AT DEHISCENCE OF BIRDSFOOT TREFOIL (*LOTUS CORNICULATUS* L.)

Introduction

Misting birdsfoot trefoil pods during seed maturation reduced seed shatter and increased seed yield (Chapter I). Seed yield was increased three-fold by twice daily mistings (3-minute periods) for 2 weeks before harvesting. However, to use a misting program effectively seed producers need a means of determining when pod dehiscence will occur in the field.

Previous studies have attempted to relate time of harvest of birdsfoot trefoil seed to specific morphological stages of growth (3, 7, 48). These studies determined the stages of pod maturation and seed development which would maximize seed yield by harvesting prior to seed pod dehiscence. Attempts to prevent pod dehiscence while immature pods ripen have not previously been recorded. In addition, various cultural practices that alter plant growth characteristics have been employed in attempts to increase seed yield (4, 14, 18, 36, 46, 47). Limited success has been achieved in increasing seed yield through implementing these various cultural practices.

There is a close interdependence between environment, moisture equilibrium, and pod shatter (4, 23, 25). Metcalfe, et al. (25) found that birdsfoot trefoil pods will dehisce readily at 30 percent relative humidity but not at 40 percent. Moisture equilibria of pods at these relative humidity levels was 10.05 and 10.39 percent, respectively.

There are several problems involved with misting of birdsfoot trefoil such as: 1) do pods with various genetic material shatter at different moisture levels, 2) at what percentage moisture should misting begin, and 3) what is the relationship of fruit, seeds, and pod moisture at dehiscence. The objectives of this study were to determine: 1) if different birdsfoot trefoil clones dehisce at the same percentage moisture, and 2) if seed producers could determine when to begin misting birdsfoot trefoil by determining pod moisture periodically.

Materials and Methods

Evaluation of Clones for Percentage Moisture at Dehiscence

Mature brown pods were randomly selected from four different clones of birdsfoot trefoil for evaluation of seed pod dehiscence in 1980. The four clones were obtained from a 50 clone, third cycle recurrent selection polycross grown in the greenhouse.

Fresh weight of individual brown pods was obtained by weighing each pod to the nearest one-tenth milligram at time of harvest. A plexiglass environmental box (L shaped) was constructed using a small squirrel cage fan and two plexiglass chambers. The box was open at both ends to provide air circulation. An external heater was placed outside of the lower portion to provide warm air (20°C) for drying pods. Small nylon mesh bags (5 x 5 cm) were constructed to hold the individual pods for dry-down and dehiscence. These bags were hung from small metal rods placed across the 3-inch width of the upper exhaust chamber.

A completely random design with 16 replications was used. At pod dehiscence, seeds and pod were removed from the environmental chamber and weighed immediately. Moisture percentage at shatter was calculated on an air dry basis.

Relationship of Fruit, Seeds, and Pod Moisture at Dehiscence

Moisture percentage of fruit, seeds, and pod was measured to determine moisture content at time of dehiscence.

Brown fruit samples were taken from 'Tretana' birdsfoot trefoil plants grown on a Bozeman silt loam (Argic-Pachic Cryoborall) soil at the Field Research Laboratory, Bozeman, MT. Samples were taken to the laboratory in a cooler and evaluated for moisture content at time of dehiscence. Brown fruit randomly selected were placed in weighing bottles and fresh fruit weights recorded. Each fruit was removed from the weighing bottle, placed in a small mesh bag, and allowed to dry on a paper towel. At time of fruit dehiscence, seeds and pod were separated, placed in separate weighing bottles, and weighed immediately. Seeds and pods were oven dried 24 hours at 100°C, cooled in a dessicator for 3 hours, and weighed.

Forty-four individual brown pods were sampled. Means, standard deviations, and standard errors were calculated based on weights and moisture percentage of fruit, seeds, and pod.

Results and Discussion

Evaluation of Clones for Moisture Percentage at Dehiscence

Birdsfoot trefoil clones did not differ in moisture percentage of brown fruit at dehiscence (Table 7), but differed in moisture percentage of fruit at harvest (Table 8) due to differences in maturity. Mean moisture percentage of air-dried fruit at dehiscence was 2.50 ± 0.28. The mean moisture percentage of air-dried fruit at harvest was 7.69 ± 0.27. Based on these data it appears that seed producers can use a critical moisture level for fruit dehiscence of birdsfoot trefoil regardless of genetic material. Rate of dry down was not determined, however its effects and importance have been substantiated by Metcalfe, et al. (25).

Relationship of Fruit, Seed, and Pod Moisture at Dehiscence

Mean moisture percentage of brown fruit at dehiscence was 7.28 ± 0.20, with a range of 4.18 - 10.00 percent. These data suggest that misting should begin when fruit moisture is approximately 10 percent. Sunlight, relative humidity, wind, temperature, and cloud cover can alter

Table 7. Moisture percentage (air dry basis) of fruit from four clones of birdsfoot trefoil at dehiscence in 1980 at Bozeman, MT.

Clone Number	Number of Fruit Sampled	Moisture Content of Fruit at Dehiscence (%)
1	16	3.167
2	16	2.625
3	16	1.894
4	16	2.300

Table 8. Moisture percentage of fresh fruit (air dry basis) of four clones of birdsfoot trefoil when placed in an environmental chamber at Bozeman, MT.

Clone Number	Number of Pods Samples	Mean Moisture Percentage of Fresh Fruit at Harvest
1	16	8.519 a*
2	16	8.106 a
3	16	6.126 b
4	16	8.026 a

*Means in the same row followed by the same letter are not significantly different by Duncan's Multiple Range Test at p = .05 level.

the dry-down rate of fruit (1, 2, 4, 49). Therefore, frequent fruit sampling is necessary to determine when misting should begin.

Moisture percentage of fruit, seed, and pod were significantly correlated with each other (Table 9). The correlation coefficient for moisture percentage of the fruit and pod at dehiscence was r = .88, indicating that fruit moisture can be used to determine when brown pods will dehisce in the field and when misting should begin.

The means, standard deviations, and standard errors for fruit, seed, and pod at dehiscence are shown in Table 10. The seed contained more moisture than the pod and fruit.

Previous work (Chapter I) indicates that misting birdsfoot trefoil twice daily increased seed production. This study indicates that seed producers should begin misting before moisture content of fruit reaches 10 percent. If environmental conditions are such that pod dehiscence will occur, the frequency of misting is dependent upon the specific environmental conditions. Under hot, dry (low relative humidity) conditions, misting must occur more frequently than under cool and wet conditions.

Table 9. Correlation matrix showing the relationship of moisture percentage of pod, seed, and fruit at dehiscence.

Percent Moisture of:	Pods at Shatter	Seeds at Shatter	Fruit at Shatter
Pods at shatter	--		
Seeds at shatter	0.47*	--	
Fruit at shatter	0.88**	0.79**	--

*,**Coefficients were significant at the .05 and .01 level of probability, respectively.

Table 10. Means, standard deviations, and standard errors of the percent moisture of pods, seeds, and fruit at dehiscence.

	$\overline{X}$	s	S.E.
Pods	6.19	1.47	0.22
Seeds	8.75	1.81	0.27
Fruit	7.28	1.67	0.20

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