The mode of reproduction in sainfoin (Onobrychis viciaefolia Scop.)
by Ronald Lynn Haaland

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Agronomy
Montana State University

Abstract:
An investigation of the mode of reproduction in sainfoin was made. Clones used in the study were from the variety Eski.

The floral morphology was found to exclude automatic self-pollination. Close examination of the style and stigma revealed appendages around the stigma that opened and closed.

Self- and cross-fertility levels as estimated by percent seed set, were re-evaluated and found to be essentially equal. Plants selfed by bees exhibited higher fertilities than plants selfed by hand.

Inbreeding studies were initiated using seedling vigor, percent fertility and number of flowers per head as measurement criteria, In general, sainfoin was not adversely affected by inbreeding.

Four plant traits were observed and proposed for potential genetic markers: plant color, petiole color, bell shaped leaf and flowers with exposed styles.

Gene exchange was not observed in reciprocal non-emasculated crosses. All evidense leads to the hypothesis that sainfoin is highly self-fertile.

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Signature Ronald Lynn Haaland

Date March 1, 1970

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

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INTRODUCTION

Sainfoin (<u>Onobrychis viciaefolia</u> Scop.) is gaining popularity in the United States and Canada as a forage crop. Breeding programs have been established to further develop sainfoins potential.

The mode of reproduction regulates plant breeding procedures. Previous literature indicates sainfoin is highly self-sterile and cross-fertile. Some observations indicated this was not the case.

The objective of this study was to thoroughly investigate the mode of reproduction in sainfoin. Studies on floral morphology, self- and cross-fertility, inbreeding, potential genetic markers and gene exchange in reciprocal crosses were conducted to accomplish this objective.

REVIEW OF LITERATURE

General

Sainfoin (Onobrychis viciaefolia Scop.) is a perennial forage legume gaining popularity in the United States because of its apparent weevil resistance and its non-bloating characteristics (10). In general, sainfoin is adapted to dryland conditions and requires a minimum of 13 inches of precipitation per year (10, 12, 24). Research in Nevada (22) has indicated that sainfoin is more winter hardy than alfalfa and the variety Eski has shown good drought and winterhardiness in Montana (15). Breeding programs have been established in the United States, Canada and Romania to further develop sainfoin's potential (8, 19, 27, 28).

Floral Morphology

The inflorescence of sainfoin is a dense spike-like indefinite raceme (head). Sainfoin has a typical legume flower with large standard and keel petals and two very small wing petals. The keel encloses ten stamens, nine united and one free, and a long style leading to an ovary containing a single ovule. The flowers open from the base of the raceme upwards to the terminal buds (26).

The sainfoin flower is pink with red viens and has a high nectar content which makes it attractive to bees (4, 14, 26). Darwin, in 1878, noted that when a sainfoin field was cut, honey bees were driven to desperation attempting to obtain nectar from red clover in an adjoining field (13).

When a bee visits a sainfoin flower, the keel is depressed and the stigma and anthers come in contact with the insect body. When the insect leaves, the keel returns to its normal position allowing other insects to trip the same flower.(26).

Fertility and Inbreeding

In 1906, Knuth reported that the style becomes more erect as anthesis progresses, ultimately projecting from the keel cleft, thus excluding automatic self-pollination and ensuring cross-pollination (23, 26).

Fleishmann, in 1932, showed that Persian sainfoin (*Onobrychis viciaefolia var. persica* Sir.) set an average of 25.5 seeds per raceme under open-pollination but when the racemes were bagged the average number of seeds per raceme was 1.5 (18, 26). Thomson hand tripped sainfoin and found fertilities of 5.11, 0.98 and 51.55 percent for tripped, untripped and open-pollinated racemes respectively. He concluded that the degree of self-fertility in sainfoin was very low (26). Self-fertility studies in Hungary show tripped fertility of 5.79 and untripped fertility of 3.74 percent (5).

Carleton reported a two year average of self-fertility in sainfoin of 6.56 and 1.91 percent for tripped and untripped respectively. Mean cross-fertility values for sainfoin and alfalfa were 26.73 and 22.55 percent respectively.

Carleton concluded from these results that alfalfa and sainfoin are similar in their average levels of self-fertility and response to tripping. Cross-fertility levels of the two crops were also similar in this study (6, 7, 8).

Hanna, in 1968, reported a mean self-fertility level in sainfoin of 4.8 percent with tripping. The mean fertility level of open-pollinated plants was reported as 35.6 percent (19).

Thomson grew selfed seed of sainfoin and found establishment to be poor and vigor reduced. There were no chlorophyll deficient plants in his material (26). Bosca and Hejja found inbreeding depressions of 26.5 and 30.2 percent for stem height and tillering respectively. They also found chlorophyll deficiency in some inbred plants (5).

Carleton found the mean inbreeding depression of seedling vigor for sainfoin and alfalfa was 29.5 and 28.1 percent respectively with one generation of selfing. He noted, however, that clones in both crops showed a range from 0 to more than 50 percent inbreeding depression (7).

Researchers working with sainfoin have concluded that sainfoin is highly self-sterile and exhibits a severe inbreeding depression upon selfing. However, evidence is accumulating which indicates that low self-fertility may not be the rule in sainfoin.

Carleton observed that seed set on plants selfed in cages by bees was much higher than would be expected from previous selfing studies (9). On isolated plants, Hanna found seed set of 1,000 to 2,500 seeds per plant. Again, this is greater than expected when earlier self-fertility estimates are taken into consideration (19).

Thomson grew two different types of sainfoin in adjacent blocks and found no gene exchange between the two types. He speculated that the lack of hybridization occurred because bees worked a small area and did not fly to other plants (26). Hanna and Carleton independently noticed lack of gene exchange in two-clone unemasculated crosses which indicates cross-fertilization did not occur (9, 20, 29).

Dubbs grew progeny rows from individual plants that were open-pollinated and was amazed at the lack of variability within individual rows (16). This lack of heterozygosity is another indication that crossing is not the general rule in sainfoin.

Hanson and co-workers found an unanticipated high degree of selfing in several two-clone crosses of alfalfa. The presence of reciprocal differences was also noted in these crosses. They concluded that additional research must be conducted to determine the extent of natural crossing in alfalfa (21).

Plant breeders have also concluded that further research must be conducted to determine the mode of reproduction in sainfoin (9, 19, 29).

Genetic Markers

Cultivated sainfoin is a polyploid having a base chromosome number of seven and a 2N number of 28. Either disomic or tetrasomic inheritance is possible but the mechanisms of inheritance have not been determined to date (7, 11). Genetic markers are essential for exact determinations of inheritance mechanisms and the extent of crossing and selfing in a crop (21). Genetic markers are slowly being accumulated in sainfoin.

The seedling leaf of sainfoin typically has one (uni), two (bi) or three (tri) leaflets. Hanna and Carleton have employed this characteristic as a genetic marker, using the different proportions of uni, bi, and tri as the measurement criterion. The type of inheritance has not been determined for this trait (7, 8, 9, 20).

White flower color has also been proposed as a genetic marker (7). The natural occurence of white flowers in sainfoin is very rare (7, 24), however, some white flowering clones have produced all white flowering progeny upon selfing (9). A genetic system for this marker has not been established but it is probably a recessive trait (9). White flower color in alfalfa is expressed by the homozygous recessive genotype (cccc) with one or more dominant alleles giving purple pigmentation of the flowers (2, 3).

Sainfoin normally has olive-green seeds, however, some plants have viable red colored seeds. The inheritance of this trait has not been characterized (7).

Carleton and Hanna have concluded that more nondeliterious genetic markers are needed to determine the mode of reproduction and inheritance in sainfoin (9, 20).

CHARACTERIZATION OF FLORAL MORPHOLOGY

Materials and Methods

An examination of floral morphology is the first step in determining the mode of reproduction of a plant. A study was initiated in February of 1969 to determine if the flower structure of sainfoin governed its mode of reproduction.

Flowers were collected at random from the variety Eski. Spacial relationship of the anthers and stigma within the flower were noted. Each flower was dissected and examined with a dissecting scope and photographed.

The sexual organs were examined more thoroughly because of their functional envolvement in reproduction. The entire style was removed from the pistle with a dissecting needle, stained with IKI, and observed with a compound microscope at 100X magnification. Photographs of the stigma were made through the microscope.

Results and Discussion

The sainfoin flower has a large standard and keel petal and two very small wing petals. The keel contains ten stamens, nine united and one free, and a long style leading to an ovary containing a single ovule (Plate 1). These results are in accordance with Thomson's findings (26).

In most flowers the style was found to protrude out of the keel cleft as anthesis progressed, however, in some flowers this was not the case. In all flowers, the style protrudes past the anthers. The

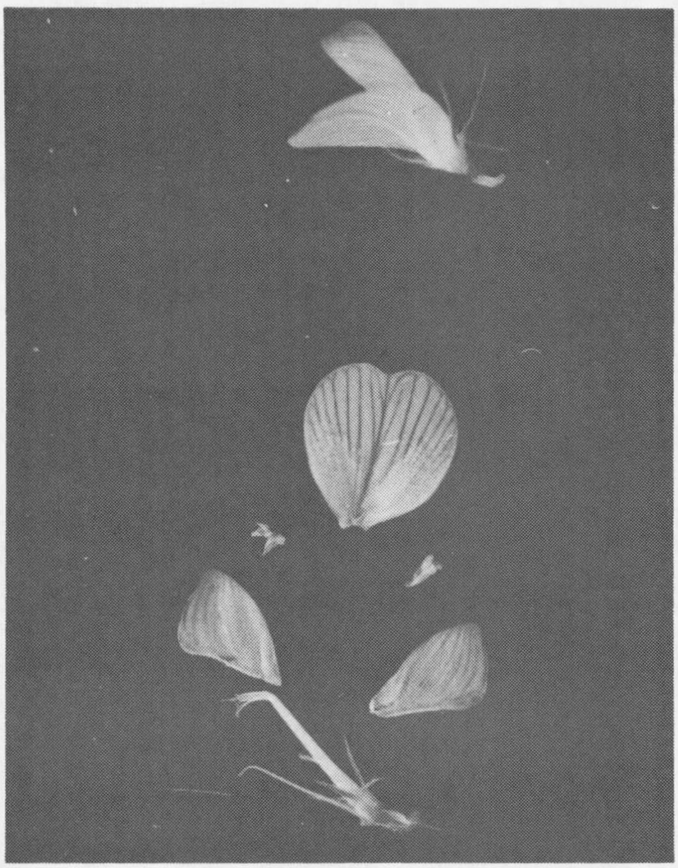

Plate 1. Intact flower above.
Disected flower below.

anthers shed thousands of pollen grains inside the keel but pollen was not observed on the stigma. This is probably due to the anther-stigma spacial relationship and indicates that automatic self-pollination is not possible in sainfoin. This does not, however, exclude the possibility of self-pollination by insects.

Microscopic observations of the female reproductive organs revealed appendages surrounding the stigma (Plate 2). The actual stigmatic surface absorbs more IKI than the appendages and is seen as the dark area inside the ring of appendages. As shown in Plates 3 and 4, the appendages may be closed over the stigmatic surface or open, exposing the stigma.

Stigmas from the flowers of different ages were examined to determine if the appendages open at a particular stage of floral development. Since sainfoin flowers open progressively from the bottom to the top of the raceme, all age groups could be obtained from a single raceme. Flowers that had reached the pink bud stage were considered very young; flowers just beginning to open, young; flowers at anthesis, mature; flowers partially closed after anthesis, old; and flowers completely wilted after anthesis, very old. The experiment was conducted on flowers from the greenhouse and flowers from the field.

A few flowers in the mature group had open appendages but the appendages were consistantly closed in all other age groups. Results from the greenhouse and the field were essentially the same. When the temperature in the greenhouse or the field became excessively warm,