FREQUENCY AND DISTRIBUTION OF ERECT GLANDULAR HAIRS ON ANNUAL MEDICAGO SPECIES AND THEIR IMPLICATIONS ON ALFALFA WEEVIL RESISTANCE AND PRELIMINARY STUDY ON POLLEN GERMINATION AND GROWTH OF POLLEN TUBES AFTER SELF AND CROSS POLLINATION IN MEDICAGO SPECIES.

by

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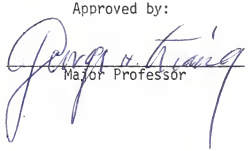

Major Professor

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M A N U S C R I P T I

FREQUENCY AND DISTRIBUTION OF ERECT GLANDULAR HAIRS ON ANNUAL *MEDICAGO* SPECIES AND THEIR IMPLICATIONS ON ALFALFA WEEVIL RESISTANCE.

Frequency and Distribution of Erect Glandular Hairs on Annual Medicago species and Their Implications on Alfalfa Weevil Resistance.

ABSTRACT

Several glandular-haired annual Medicago species are highly resistant to two destructive alfalfa insects: alfalfa weevil Hypera postica Gyllenhal} and potato leafhopper {Empoasca fabae (Harris)}. To aid us in elucidating the resistance mechanism, we studied the frequency and distribution of glandular hairs on two tetraploid (M. rugosa, M. scutellata) and two diploid (M. blancheana, M. disciformis) species. The four annual species have the erect capitate glandular hairs on all vegetative and floral parts except the cotyledons and flower petals. Hair density differed on plants grown in field and greenhouse, and plant parts and species reacted differentially. Density was not affected by ploidy level. Hair density was greatest on the peduncle followed by the stem, petiole, and least on the pods and leaflets.

The diploids and tetraploids vary in the location of hairs on their leaves. Both have glandular hairs on leaflet margins, but on tetraploids, the hairs are distributed over the entire abaxial surface; on diploids, they are confined to the midrib area. When averaging the glandular hairs over all organs, M. scutellata (Turkey) has the greatest hair density followed in order by M. scutellata (Australia), M. scutellata (Argentia), M. disciformis, M. blancheana and M. rugosa.

Additional index words: Taxonomy, Cytology, Procumbent glandular hairs, Medicago sativa.

INTRODUCTION AND LITERATURE REVIEW

Most plants have outgrowths called hairs or trichomes. There are two types of hairs in Medicago species (simple and glandular). In most plants the hairs vary in density from one organ to another and they occur in various forms and sizes (Levin 1973).

Certain Medicago species have only simple hairs whereas some have both simple and the glandular hairs. The glandular-haired species are resistant to the first instar larvae of alfalfa weevil {Hypera postica (Gyllenhal)} and to the piercing insect potato leafhopper {Empoasca fabae (Harris)}. Plants of Medicago sativa L. subsp. sativa, the commercially grown alfalfa, lack the upright but contain procumbent glandular hairs (Wilson 1913). Preliminary information indicates that the procumbent glandular hairs do not provide resistance to the larvae of alfalfa weevil (Johnson and Sorensen 1978, Unpublished data).

Barnes and Ratcliffe (1969) detected five annual species of Medicago which have moderate resistance to the alfalfa weevil. Three of them possess the glandular hairs (M. rugosa Desr., M. scutellata and M. minima). Shade et al. (1975) demonstrated that both M. disciformis and M. scutellata have glandular hairs present at high densities equally on stems and petiole. The older plants have more glandular hairs than younger ones. The readings on the stem for M. disciformis for the mean hair density, mean hair length, and mean head diameter are $12.3 \pm 1.3/mm^2$, $263.7 \pm 1.9 \mu m$ and $30.5 \pm 1.2 \mu m$, respectively.

Heyn (1963) mentioned the location of the glandular hairs on annual of Medicago species but not their frequency. The distribution of the glandular hairs did provide a taxonomic criterium. The glandular hairs

could be used also as genetic markers for the incorporation of a resistant trait into a quality plant. Lesins (1969) studies the inheritance of the glandular hair trait on pods in Medicago. He found that the F_2 generation after crossing M. hybrida Trantv. (without glandular hairs on pods) with M. suffruticosa Ram. (with glandular hairs on pods) showed a 3:1 ratio of no hairs to hairiness was observed. The presence of glandular hairs on the pods in the diploid alfalfa were inherited as a simple recessive trait.

The role of glandular hairs as a defense against insects is obvious. It can affect the ability of the insects to function through both mechanical and chemical mechanisms (Johnson 1968; Levin 1973). The glandular hairs were observed to have a bulbous head that secretes exudates that may be toxic or offensive to the would be predators. Uphof (1962) reviewed the hair and the glandular structures of many plant species.

Much research has been done to identify the significant relationship of hairs to insect resistance. Levin (1973) reviewed information regarding involvement of glandular hairs in the biochemical defense against insects. The exudates from glandular hairs can provide three types of defense mechanisms: 1) trapping, 2) poisoning, and 3) gustatory or olfactory repellents. Thurston, Smith and Cooper (1966) reported that the alkaloids produced by glandular hairs of Nicotiana species are toxic to green peach aphid which may be a serious pest. Thurston (1970) indicated that alkaloid compounds are particularly abundant in glandular hairs of Nicotiana and Petunia and these products are highly toxic to larvae of tobacco hornworm. Gibson (1971) reported

the trapping mechanisms of the glandular hair exudates. The exudates immobilized the green peach aphids when it contacts the hairs, thus it starves the insects to death. These nonvolatile hair exudates such as alkaloids and simple or complete phenolics may serve as gustatory or olfactory repellents. Matsumoto (1962) reported that the coumarin produced by Melilotus species has an inhibitory effect on the feeding of vegetable weevil. Tannins are responsible for the bitter or astringent taste of many plant tissues. Feeny (1968, 1969) has demonstrated the deleterious effect of tannins on the larval growth of the winter moth.

Like most plants, the exudates from the glandular hairs of certain Medicago species have not been analysed systematically for its defensive properties. Shade et al. (1975) suggested that death of alfalfa weevil larval on glandular haired M. disciformis and M. scutellata is of a mechanical nature rather than a toxic reaction. Many dead larvae were found glued to the stems.

The alfalfa weevil has been one of the nation's most destructive alfalfa insect pests. Though insecticides can effectively control most insects, the use of genetically resistant plants is much desired in terms of economic and ecological point of view. Several glandular-haired annual Medicago species are highly resistant to two destructive alfalfa insects (alfalfa weevil and potato leafhopper). The purpose of this research is to aid in elucidating the resistance mechanism and also for taxonomic study. The frequency and distribution of glandular hairs on two tetraploid (M. rugosa, M. scutellata) and two diploid (M. blancheana, M. disciformis) annual Medicago species are investigated.

MATERIAL AND METHODS

Four annual glandular-haired Medicago species were selected to study the frequency and distribution of the glandular hairs. They were two diploids (2x=16): M. disciformis and M. blancheana, and two tetraploids (4x=32): M. rugosa and M. scutellata. For M. scutellata three seed sources were used: Argentina, Australia and Turkey.

Approximately 30 seeds for each entry were hand scarified and germinated on blotting paper in petri dishes in the growth chamber at temperature 21.1°C ± 1°C. The germinated seeds were then transfered to 5x5 cm. peat pots in Jiffy mix. After 4 to 5 weeks they were transplanted to soil in 10 cm. clay pots in the greenhouse under ambient light conditions. After 2 weeks in the greenhouse, 15 plants from each species or sources were transplanted in the field at Ashland. The other 15 plants from each entry were left in the greenhouse.

Observations under two conditions were made: greenhouse and field. The study on the frequency and distribution of the glandular hairs were made at first flower. In each species or sources, four plants were randomly selected for observation. Selected branch from each randomly selected plant was analysed for the glandular hairs at three positions: base, middle, apex. Observations were made on the stem, petiole, adaxial surface of the leaflets, peduncle, sepals and pods. On the abaxial surface of the leaflet, counts were made at the margin and the region around the midvein.

The parts to be studied were first washed with tap water and then dipped in a 50% solution of water soluble aniline blue. The aniline blue solution helps give a clear view of the glandular hairs.

The frequency of the glandular hairs were determined by counting them directly under the dissecting microscope at a magnification of 16X. A micrometer was used for the measurement of the area to be observed.

For all samples, the frequency and distribution of the glandular hairs were statistically analysed for both the greenhouse and field observations.

The glandular hairs from each species or subspecies were examined under the scanning electron microscope. We examine the morphology and the structure of the glandular hairs. The procedure for preparing the specimen for observation under SEM was based on Liang and Sorensen (1978).

RESULTS AND DISCUSSIONS

The four annual Medicago species (M. scutellata, M. blancheana, M. rugosa and M. disciformis) have glandular and simple hairs. The upright glandular hairs are morphologically similar for all the four species as seen under the scanning electron microscope (Fig. 1A). The glandular hairs are tough and erect, having enlarged glandular heads at the tip of the stalk, which is the site of the glandular exudates. M. sativa L. has the procumbent type of glandular hairs (Fig. 1B).

The glandular hairs occur on the stipules, petioles, leaflets, stems, peduncle, pods and sepals. No glandular hairs are present on the cotyledons or the petals of these four species. The distribution of the glandular hairs on the abaxial or adaxial surface of the leaflets are affected by ploidy level.

The diploids (M. blancheana and M. disciformis) have glandular hairs at the margin of the adaxial surface of the leaflet. The abaxial side has glandular hairs at the margin and on the midribs only. Simple hairs are found on both surfaces of the leaflets.

The tetraploids (M. scutellata and M. rugosa) have glandular hairs on the margin of the adaxial surface of the leaflets. The abaxial surface have glandular hairs over the entire surface including the edges and the midribs. Simple hairs are found only on the adaxial surface.

There is no significant difference among the means of the glandular hairs for the plants within species. This is expected because all the four species are self-pollinated species and therefore plants

within species are homogeneous genetically. There is significant difference between the environments (greenhouse and field) for the five organs examined (petiole, adaxial surface of leaflet, abaxial surface of leaflet, stem, and pod) but not significant for the peduncle. There are significant differences among the organs within species as well as between species.

Greenhouse Environment

Table 1 indicates the frequency of the glandular hairs on the organs of the various species. There is significant difference for the density of glandular hairs among the species (for all the organs), positions, and also among organs within species. M. disciformis (725/cm.^{2}) has the highest number of glandular hairs on the petiole. Second is M. scutellata (Turkey) (650/cm^2), and is followed by M. scutellata (Argentia) (555/cm.^{2}),M. blancheana (545/cm.^{2}), M. scutellata (Australia) (488/cm.^{2}) and M. rugosa (466/cm.^{2}). For the adaxial surface of the leaflet, M. disciformis (227/cm.^{2}) has the highest frequency and is followed by M. scutellata (Turkey) (214/cm.^{2}), M. blancheana (197/cm.^{2}), M. rugosa (170/cm.^{2}), M. scutellata (Argentina) (166/cm.^{2}) and M. scutellata (Australia) (162/cm.^{2}). The tetraploid species have more glandular hairs than the diploid on the abaxial surface of leaflets and this has been explained earlier. M. rugosa (606/cm.^{2}) and M. scutellata (Turkey) (601/cm.^{2}) has more glandular hairs than the others. This is followed by M. scutellata (Argentina) (478/cm.^{2}), M. scutellata (Australia) (482/cm.^{2}), M. disciformis (334/cm.^{2}) and least is M. blancheana (233/cm.^{2}) (233/cm.^{2}). For the stem M. scutellata (Turkey) (1091/cm.^{2}) has the highest number of glandular hairs

followed by M. scutellata (Argentina) (928/cm.^{2}), M. scutellata (Australia) (863/cm.^{2}), M. blancheana (605/cm.^{2}), M. disciformis (535/cm.^{2}) and M. rugosa (356/cm.^{2}).

M. scutellata (Australia) (2070/cm.^{2}) ranks first in the glandular hairs density for the peduncle. M. scutellata (Turkey) (2010/cm.^{2}) is second followed by M. scutellata (Argentina) (1870/cm.^{2}), M. disciformis (1520/cm.^{2}) M. blancheana (835/cm.^{2}). and M. rugosa (790/cm.^{2}). For the pods once again M. scutellata (Turkey) (401/cm.^{2}) ranks first in the glandular hairs density. This is followed by M. scutellata (Australia) (373/cm.^{2}), M. scutellata (Argentina) (323/cm.^{2}), M. disciformis (326/cm.^{2}), M. blancheana (137/cm.^{2}) and M. rugosa (119/cm.^{2}).

The tetraploid species have more glandular hairs than the diploid on the abaxial surface of leaflet only. Overall M. scutellata has more glandular hairs than the other species for the petiole, abaxial surface of the leaflet, stem, peduncle and pods. When averaging the glandular hairs over all organs, M. scutellata (Turkey) has the most hairs compared to the others. Second is M. scutellata (Argentina) followed by M. scutellata (Australia), M. disciformis, M. blancheana and finally M. rugosa.

There is not much difference in the frequency of the glandular hairs of the organs (petiole, adaxial surface leaflet, abaxial surface leaflet and stems) among positions (base, middle and apex) within a species (Table 2, 3, 4, 5). The apex position usually has the most glandular hairs when compared to the middle or base positions. This is because the tissues at the apex have not fully elongated. M. rugosa has some of the glandular hairs broken at the base position

probably due to aging and this explains why the base position has less hair count than the middle or apex position.

Comparison of the hair density on organs within species indicate that they are significantly different between each other. M. scutellata (Turkey), M. scutellata (Argentina) and M. scutellata (Australia) have the highest number of glandular hairs on the peduncle, followed by the stem and the petiole. M. rugosa has the highest number on the peduncle followed by the adaxial surface of the leaflet, petiole and stem. M. disciformis has the highest number of hairs on the peduncle followed by the petiole and stem. Overall, all the species have the highest number of glandular hairs on the peduncle and the least on pods and the adaxial surface of leaflets.

Field Environment

There is a significant difference in the glandular hairs between the species for the organs, positions and also the organs within species. Table 6 indicates the frequency of the glandular hairs on the organs of the various species. M. scutellata (Argentina) (922/cm.^{2}) has the highest number of glandular hairs on the petiole hairs followed by M. blancheana (843/cm.^{2}), M. scutellata (Australia) (840/cm.^{2}). M. scutellata (Turkey) (803/cm.^{2}), M. rugosa (698/cm.^{2}) and M. disciformis (666/cm.^{2}). For the adaxial surface of leaflet, M. blancheana (843/cm.^{2}) has the highest number of glandular hairs followed by M. scutellata (Australia) (175/cm.^{2}), M. rugosa (162/cm.^{2}) M. scutellata (Turkey) (160/cm.^{2}), M. scutellata (Argentina) (158/cm.^{2}) and M. disciformis (143/cm.^{2}). There is not much difference between species in the means of the glandular hairs on the adaxial surface of leaflet because they are found only

along the margins.

The tetraploids (M. scutellata and M. rugosa) have more glandular hairs on the abaxial surface of the leaflet than do the diploids (M. blancheana and M. disciformis). M. scutellata (Australia) (510/cm.^{2}) has the highest number of glandular hairs followed by M. ruguosa (478/cm.^{2}), M. scutellata (Turkey) (456/cm.^{2}), M. scutellata (Argentina) (401/cm.^{2}), M. blancheana (177/cm.^{2}) and M. disciformis (163/cm.^{2}).

M. scutellata has more hairs on the stem than do the other species. M. scutellata (Australia) (1270/cm.^{2}) has the highest number of hairs on the stem and is followed by M. scutellata (Turkey) (1265/cm.^{2}), M. scutellata (Argentina) (1066/cm.^{2}), M. blancheana (870/cm.^{2}), M. disciformis (670/cm.^{2}) and M. rugosa (516/cm.^{2}). For the peduncle, M. scutellata (Turkey) (1915/cm.^{2}) has the most glandular hairs. M. disciformis (1620/cm.^{2}) is second and followed by M. scutellata (Australia) (1475/cm.^{2}), M. blancheana (1215/cm.^{2}) and M. rugosa (525/cm.^{2}). However, there is no reading for the M. scutellata (Argentina) because they did not flower, probably due to the environmental factors such as photoperiod effect.

M. scutellata (Turkey) (506/cm.^{2}) has the highest number of glandular hairs for the pods. M. disciformis (412/cm.^{2}) is second followed by M. scutellata (Australia) (365/cm.^{2}), M. blancheana (242/cm.^{2}) and M. rugosa (146/cm.^{2}). There is no reading for M. scutellata (Argentina).

Overall M. scutellata has more glandular hairs than the rest for the organs: stem, peduncle, and pods. Based on the average total of all the organs, M. scutellata (Turkey) has the most glandular hairs and is followed by M. scutellata (Australia), M. disciformis, M. blancheana and M. rugosa {excluding M. scutellata (Argentina)}.

Table 7, 8, 9, 10 indicates the glandular hairs on the organs at certain positions. There is not much difference in the means of the glandular hairs from position to position within a species. Most of the organs have less glandular hairs at the base position or the middle as compared to the apex. This is because the organs at the apex have not fully elongated. As for the stem, the base position has lesser glandular hairs compared to the rest of the two positions. Aging and its relation near the ground has some effect on the breaking off some of the glandular hairs.

There are significant differences in the means of the glandular hairs of the organs within a species. M. scutellata (Turkey), M. scutellata (Australia), M. blancheana and M. disciformis have most glandular hairs on the peduncle followed by the stem and petiole. However, M. rugosa has the most glandular hairs on the petiole and peduncle followed by the stem, abaxial leaf surface and adaxial leaf surface. The tetraploids (M. scutellata and M. rugosa) have the least number of glandular hairs on the pods and the adaxial leaf surface. The diploids have the least on the adaxial and abaxial surface of leaflets and pods.

There is a significant difference in the density of the glandular hairs at the various organs betweeen the two environments. For M. scutellata (Turkey) there is a significant difference between the environments for all the organs (petiole, stem, adaxial and abaxial surface of leaflet) except the flower stalk. There is no significant difference between the two environments for the species M. scutellata (Australia) in the adaxial surface leaflets and pods. M. blancheana

showed a significant difference in petiole, abaxial surface leaflet, stem, peduncle and pods. M. rugosa showed a significant difference in petiole, abaxial leaflet surface and stem. M. disciformis showed a significant difference in the adaxial, abaxial leaflet surface, stem and pods. On the whole for all the species, a greater difference in means between the two environments was found in the petiole, stem, and pods.

Summarizing the data from both environments, the M. scutellata have the most glandular hairs compared to the rest of the species. M. scutellata (Turkey) has the most followed by M. scutellata (Australia), M. scutellata (Argentina), M. disciformis, M. blancheana and M. rugosa. The peduncle ranks first in the organs that have the most glandular hairs. Second is the stem followed by the petiole and least are pods, abaxial and adaxial leaflet surface.

The frequency and distribution of the glandular hairs can thus explain the defense mechanisms of annual Medicago species to alfalfa weevil larvae. After the eggs have hatched, the larvae in searching for food and shelter crawl upwards passing the stem. They have to pass the petiole in order to get to the leaflet. Once in the leaflet they find shelter in the abaxial surface so as to escape from predators, and adverse environments. This would probably explain why the tetraploids have glandular hairs on the abaxial surface of the leaflet and the diploids have on the margin and midrib. Neither the tetraploid nor diploid have glandular hairs on the adaxial surface of the leaflet except at the margins.

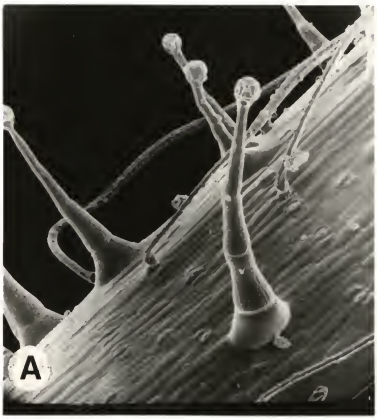
In the process of moving upwards the larvae gets confused due to the

hinderance effect of the glandular hairs. The sticky exudate of the glandular hairs make their movement limited. The combination of these two effects thus result in their death (Sorensen, E. L. Unpublished data 1978).

Figure 1.

A: Glandular hairs (erect) on stem of *M. rugosa*.

B: Glandular hairs (procumbent) on stem of *M. sativa* L.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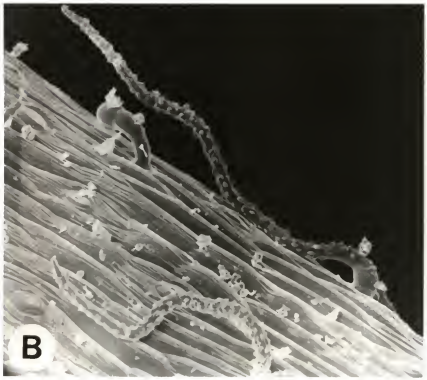
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Table 1. Mean values of the glandular hairs (number per sq. cm.) on plant parts of *Medicago* species grown in the greenhouse.

	Petiole	Adaxial surface of leaflet	Abaxial surface of leaflet	Stem	Peduncle	Pods
M. scutellata (Turkey)	650 a[z]	214 ab[z]	601 a[z]	1091 a[z]	2010 a[z]	401 a[z]
M. scutellata (Argentina)	555 b	166 c	478 b	928 b	1870 a	323 b
M. scutellata (Australia)	488 b	162 c	482 b	863 b	2070 a	373 ab
M. blancheana	545 b	197 b	233 d	605 c	835 c	137 c
M. rugosa	466 b	170 c	606 a	356 d	790 c	119 c
M. disciformis	725 a	227 a	334 c	535 c	1520 b	326 b

[z] Mean separation by Duncan's multiple range test, 5% level.

Table 2. Mean values of the glandular hairs (number per sq. cm.) on three positions of petiole of Medicago species in the greenhouse.

	Positions			
	Base	Middle	Apex	Average
M. scutellata (Turkey)	800	535	615	650 a[z]
M. scutellata (Argentina)	610	510	615	555 b
M. scutellata (Australia)	465	465	535	488 b
M. blancheana	520	435	680	545 b
M. rugosa	470	445	485	466 b
M. disciformis	690	750	735	725 a

[z] Mean separation by Duncan's multiple range test, 5% level.

Table 3. Mean values of the glandular hairs (number per sq. cm.) on three positions on adaxial of surface of leaflet of Medicago species in the greenhouse.

	Positions			Average
	Base	Middle	Apex	
M. scutellata (Turkey)	212	200	231	214 ab[z]
M. scutellata (Argentina)	175	156	168	166 c
M. scutellata (Australia)	168	156	162	162 c
M. blancheana	193	175	225	197 b
M. rugosa	181	175	156	170 c
M. disciformis	218	200	262	227 a

[z] Mean separation by Duncan's multiple range test, 5% level.

Table 4. Mean values of the glandular hairs (number per sq. cm.) on three positions of abaxial surface of leaflet of Medicago species in the greenhouse.

	Positions			
	Base	Middle	Apex	Average
M. scutellata (Turkey)	759	550	494	601 a[z]
M. scutellata (Argentina)	491	441	503	478 b
M. scutellata (Australia)	465	453	528	482 b
M. blancheana	237	218	244	233 d
M. rugosa	669	647	503	606 a
M. disciformis	309	297	397	334 c

[z] Mean separation by Duncan's multiple range test, 5% level.

Table 5. Mean values of the glandular hairs (number per sq. cm.) on three positions of stem of Medicago species in the greenhouse.

	Positions			
	Base	Middle	Apex	Average
M. scutellata (Turkey)	1105	990	1180	1091 a[z]
M. scutellata (Argentina)	965	800	940	928 b
M. scutellata (Australia)	975	800	815	863 b
M. blancheana	635	655	525	605 c
M. rugosa	245	310	515	356 d
M. disciformis	505	510	590	535 c

[z] Mean separation by Duncan's multiple range test, 5% level.

Table 6. Mean values of the glandular hairs (number per sq. cm.) on plant parts of Medicago species grown in the field.

	Petiole	Adaxial surface of leaflet	Abaxial surface of leaflet	Stem	Peduncle	Pods
M. scutellata (Turkey)	803 bc[z]	160 ab[z]	456 b[z]	1265 a[z]	1915 a[z]	506 a[a]
M. scutellata (Argentina)	926 a	158 ab	401 c	1066 b	----	---
M. scutellata (Australia)	840 abc	175 a	510 a	1270 a	1475 bc	365 b
M. blancheana	843 a	179 a	177 d	870 c	1215 c	242 c
M. rugosa	698 d	162 ab	478 ab	516 e	525 d	146 d
M. disciformis	666 d	143 b	163 d	670 d	1620 ab	412 b

[z] Mean separation by Duncan's multiple range test, 5% level.

Table 7. Mean values of the glandular hairs (number per sq. cm.) on three positions of petiole of Medicago species in the field.

	Positions			
	Base	Middle	Apex	Average
M. scutellata (Turkey)	700	830	880	803 bc[z]
M. scutellata (Argentina)	725	885	1170	926 a
M. scutellata (Australia)	775	860	885	840 abc
M. blancheana	775	940	815	843 a
M. rugosa	700	715	680	698 d
M. disciformis	580	645	775	666 d

[z] Mean separation by Duncan's multiple range test, 5% level.

Table 8. Mean values of the glandular hairs (number per sq. cm.) on three positions of adaxial surface of leaflet of Medicago species in the field.

	Positions			Average
	Base	Middle	Apex	
M. scutellata (Turkey)	156	168	156	160 ab[z]
M. scutellata (Argentina)	125	175	175	158 ab
M. scutellata (Australia)	162	175	187	175 a
M. blancheana	150	162	225	179 a
M. rugosa	162	150	175	162 ab
M. disciformis	118	156	156	143 b

[z] Mean separation by Duncan's multiple range test, 5% level.

Table 9. Mean values of the glandular hairs (number per sq. cm.) on three positions of abaxial surface of Medicago species in the field.

	Positions			
	Base	Middle	Apex	Average
M. scutellata (Turkey)	328	484	556	456 bc[z]
M. scutellata (Argentina)	309	387	506	401 c
M. scutellata (Australia)	437	531	562	510 a
M. blancheana	144	140	247	177 d
M. rugosa	478	528	428	478 ab
M. disciformis	141	165	184	163 d

[z] Mean separation by Duncan's multiple range test, 5% level.

Table 10. Mean values of the glandular hairs (number per sq. cm.) on three positions of stem of Medicago species in the field.

	Positions			
	Base	Middle	Apex	Average
M. scutellata (Turkey)	900	1250	1645	1265 a[z]
M. scutellata (Argentina)	755	1170	1275	1066 b
M. scutellata (Australia)	980	1350	1475	1270 a
M. blancheana	705	770	1135	870 c
M. rugosa	450	500	600	516 e
M. disciformis	595	655	760	670 d

[z] Mean separation by Duncan's multiple range test, 5% level.

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M A N U S C R I P T II

PRELIMINARY STUDY ON POLLEN GERMINATION AND GROWTH OF POLLEN TUBES AFTER SELF AND CROSS POLLINATION IN MEDICAGO SPECIES.

Preliminary Study on Pollen Germination and Growth of Pollen Tubes After Self and Cross Pollination of Medicago species.

ABSTRACT

Pollen tubes growth in the style of the Medicago species was observed by use of fluorescence microscopy. The tissues of the style of M. scutellata (annual) are softer than those of M. sativa L. (perennial). M. scutellata required 17 hours for a good softening in 1N NaOH whereas M. sativa needed more than 24 hours. For softening process less time is required if a higher normality of NaOH was used.

In the attempted interspecific cross between M. sativa and M. scutellata, entry of some pollen tubes into the ovule was observed. Most pollen did not germinate; however a few did but most of the pollen tubes did not penetrate into the style. M. scutellata have the pollen diameter range from 0.032 - 0.046 mm. and M. sativa ranging from 0.042 - 0.056 mm. However most pollen of M. sativa have the same size as pollen of M. scutellata. No seeds were produced from this attempted cross.

INTRODUCTION

Several glandular-haired annual Medicago species are resistant to two destructive alfalfa insects: alfalfa weevil {Hypera postica (Gyllenhal)} and potato leafhopper {Empoasca fabae (Harris)}. The commercial alfalfa, M. sativa L. subsp. sativa have procumbent but not upright glandular hairs present in some of the annual Medicago species. Preliminary information indicates that the procumbent glandular hairs do not provide resistance to the larvae of the alfalfa weevil (Johnson and Sorensen 1978, Unpublished data). Therefore interspecific crosses are necessary to transfer upright glandular hairs to hay types. Elgin et.al. (1977) attempted crosses between glandular-haired M. scutellata and M. sativa but produced no hybrid.

Kho and Baer (1970) carried out a microscopical investigation to find the causes of the failure of the cross between scaled and unscaled Rhododendron species. To observe the growth of pollen tubes into the style and the ovary, a special fluorescence microscope was used.

Fluorescence microscopy has been well accepted for use in the study of pollination incompatibility. The procedure is based on the selective uptake of certain fluorescent substances called fluorochromes by the cells. The technique depends on the occurence of callose which lines and plugs pollen grains and pollen tubes (Martin 1958). Currier (1957) demonstrated that callose in either living or dead tissue can be stained selectively with water soluble aniline blue and similar dyes which fluorescent in the ultra violet light.

The objective of this research was to study the incompatibility problem in the crosses between M. scutellata and M. sativa L. subsp. sativa.

Also this research was conducted to find the proper technique so pollen tube growth in the style and ovary can be studied.

MATERIALS AND METHODS

In this study two tetraploid Medicago species were used, perennial M. sativa L. subsp. sativa and annual M. scutellata. Twenty seeds from M. scutellata were hand scarified and germinated on blotter paper in petri dishes in the growth chamber at temperature 21.1°C±1°C. The germinated seeds were transfered to 5X5 cm. peat pots in Jiffy mix. After 4 to 5 weeks, they were transplanted to soil in 10 cm. clay pots in the greenhouse. Fifteen M. sativa L. subsp. sativa plants which were already in the greenhouse were used.

We observed the pollen germination and pollen tube growth in the selfed M. sativa, selfed M. scutellata, M. sativa x M. scutellata and reciprocals (with or without emasculation of the female flower). Observations were made 24 hours after pollination.

For selfing the plants, the flower was tripped with a toothpick. When crossing, the plants were emasculated prior to pollination to avoid selfing. The suction method (Kirk 1930) was used to remove anthers and pollen in the female flower. The standard petal was first clipped and the flower was gently tripped. This suction method was applied with glass tubing drawn to 1 mm. tip and inserted in a rubber hose attached to a vacuum source. A low powered binocular magnifier (16X) was used to determine the thoroughness of pollen removal.

The flowers were first fixed in 1 (formalin): 8 (80% alcohol): 1 (glacial acetic acid) for 24 hours or more. After rinsing in distilled water, they were put in a series of aqueous solution of NaOH from 1 N to 6 N for a period from 1 to 24 hours. This study was made in order to find out the best combination of the normality of NaOH

with time needed to soften the tissue and to permit adequate penetration of the dye. The softened flower was transfered to a petri dish of distilled water for 15 minutes to remove most of the NaOH. The flowers were then stained in 0.1% solution of water soluble aniline blue dye in 0.1 N K_3PO_4 for 5 min.

For observation of the style, the sepals and petals were removed carefully with a probe needle. The styles were mounted in a few drops of the staining solution on a clean glass slide and were covered with clover slips. The styles were then carefully crushed by tapping the cover slip with a probe needle directly above the style. The styles were then ready for observation under the fluorescence microscope. For storage of the slides, finger nail polish was used to cover the lining between the coverslip and the glass slide.

The pollen from each species (M. sativa and M. scutellata) were treated with acetocarmine and was observed under the light microscope (15X). The size, diameter, and the morphology of the pollen were studied.

RESULTS AND DISCUSSIONS

Growth of pollen tubes in the style of M. sativa L. and M. scutellata were easily seen under the fluorescence microscope. The callose in the pollen tubes fluoresced distinctly and was observed laid down in closely spaced plugs (Fig. 1). Some callose was seen in the sieved tubes and in the epidermal simple hairs of the style. However, they were easily distinguished from the pollen tubes by their size, shape and distribution in the style.

Treatment of the style of M. sativa L. in 1N NaOH for 24 hours was best for softening the tissues and for permitting adequate penetration of the dye. However, M. scutellata needed 17 hours for the treatment in order for best softening of the tissue. Proper softening of the tissues was important because this would affect the performance of the slide for viewing the pollen tubes. If the styles were too soft, the tissues would be smashed when making the slide. If the style was not soft enough, then it could not be easily neatly smashed.

The tissues of M. scutellata (annual) were softer than those of the M. sativa (perennial). M. scutellata needed less time for the softening process. Sayers and Murphy (1966) treated M. sativa from 24 to 36 hours in 1N NaOH for the study on the pollen tube growth, fertilization frequency and post fertilization ovule abortion as related to seed set. However, both M. sativa and M. scutellata could be softened with lesser time if a higher normality of NaOH was used. Table 1 indicates the best combination of the normality of NaOH and the time needed to treat the styles of M. scutellata in order to give a good softening of the tissues.

All the pollen on the stigma of selfed M. scutellata and self compatible M. sativa germinated (Fig. 1). However, a few pollen tubes were observed in the style indicating that not all the germinating pollen penetrated the stigma.

In the attempted interspecific cross between M. sativa L. (male) and M. scutellata (female) did not produce seeds. Studies on pollen tube growth indicated that 45 out of 70 pollinations, showed no pollen tube growth in the style. This category included pollen that did not germinate and pollen that germinated but did not penetrate the stigma (Fig. 2). In other words, 35.7% of the pollinations showed pollen tube growth in the style. Some of these pollen were observed entering the ovules (Fig. 2).

In the reciprocal cross between M. sativa and M. scutellata 16 out of 20 pollinations lacked pollen tube growth in the style. The rest did have pollen tube growth in the style.

The pollen from M. scutellata had a diameter ranging from 0.032 - 0.046 mm. (Fig. 3) whereas diameter of M. sativa ranged from 0.036 - 0.056 mm. (Fig. 3). Most of the pollen from M. sativa have diameter ranged from 0.042 - 0.046 mm. In other words, most of the pollen of M. sativa have a size similar to those of M. scutellata.

Pollen from both species (M. scutellata and M. sativa) were used in the pollination of M. sativa and M. scutellata. However, all the pollen (germinated and ungerminated) on the stigma and pollen tubes in the style could not be distinguished whether they were M. scutellata or M. sativa. Therefore it was difficult to tell whether those pollen tube as seen in the styles of the attempted cross between M. sativa

and M. scutellata were due to self or contamination.

At present time, the incompatibility that seems to occur in this attempted cross (M. sativa X M. scutellata) seems to be located in stigma. Some pollen germinated however most of the growth ceased in the stigma. For those pollen tubes that grow in the style, some were observed entering the ovules. There was no indication of incompatibility behaviour of the pollen tube once in the style. Coiling of the pollen tubes or broken pollen tube tips as occured in the Rhododendron hybridization (Kho and Baer, 1973) was not observed.

The attempted crosses did not produce seeds. The result indicates that some fertilization occurs in the crosses. Probably there is not enough endosperm to keep the embryo to develop into a mature seed. The flowers fall to the ground a few days after the pollination was made. Therefore if this is the case, embryo culture is the best possible means of getting a hybrid. If this interspecific incompatibility is due to complete incompatibility, use of bridging species, plant hormones, or removing the stigmatic surface by cutting off the stigma and pollination of the cut surface may be worth trying for obtaining a hybrid.

Table 1. Combination of N NaOH and time needed for softening the styles of *M. scutellata*.

Normality of NaOH	Time (Hours)
1	17
2	15
3	10
4	7
5	5
6	4

Figure 1.

A: Pollen tubes on self M. sativa L.

B: Upright glandular hairs and simple hairs (fluoresce) on outer tissues of the ovary of M. scutellata.

C: Procumbent glandular hairs on outer tissues of the ovary of M. sativa.

D: Pollen germination on stigma of self M. scutellata.

A
B
C
D

Figure 2. Attempted interspecific cross, *M. sativa* X *M. scutellata*.

A: No pollen germination on stigma of *M. scutellata*.

B: A pollen germinates on stigma of *M. scutellata*.

C: Pollen tube growth in style of *M. scutellata*.

D: Entry of a pollen tube into the ovule of *M. scutellata*.

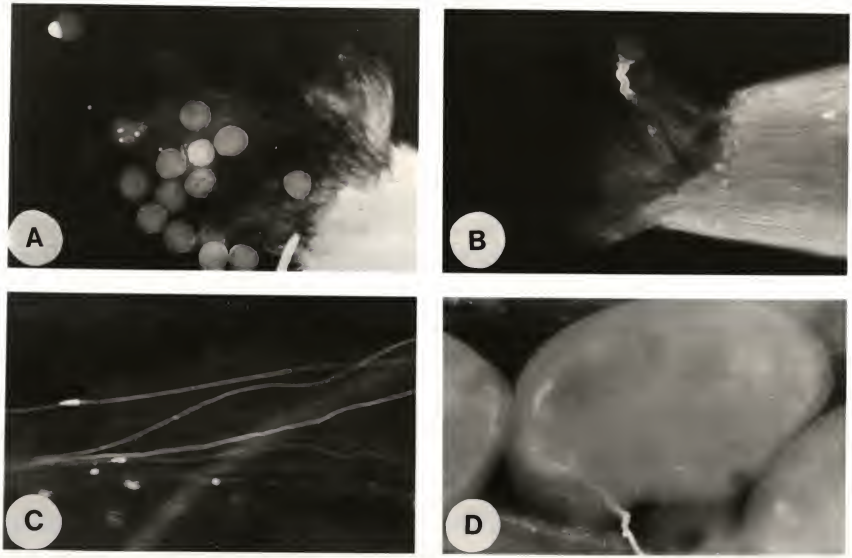
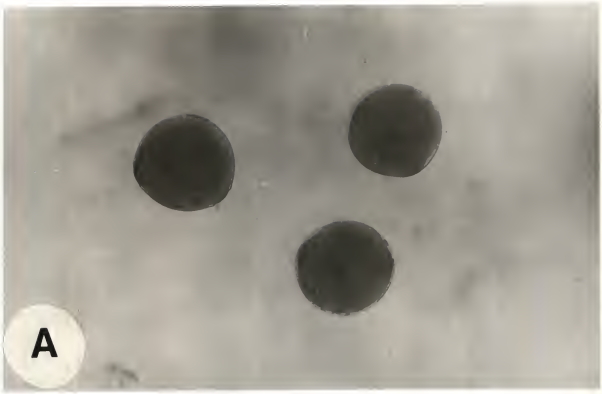
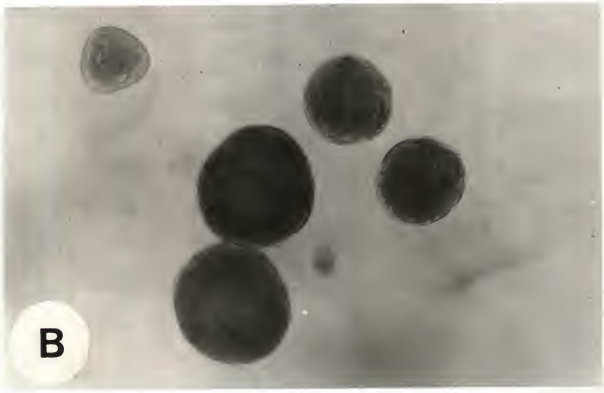

A
B
C
D

Figure 3.

A: Pollen of *M. scutellata*. 150 X.

B: Pollen of *M. sativa*. 150 X.


A


B

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FREQUENCY AND DISTRIBUTION OF ERECT GLANDULAR HAIRS OF ANNUAL MEDICAGO SPECIES AND THEIR IMPLICATIONS ON ALFALFA WEEVIL RESISTANCE AND PRELIMINARY STUDY ON POLLEN GERMINATION AND GROWTH OF POLLEN TUBES AFTER SELF AND CROSS POLLINATION IN MEDICAGO SPECIES.

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

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MASTER OF SCIENCE

Department of Agronomy
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Manhattan, Kansas

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ABSTRACT

Several glandular-haired annual Medicago species are highly resistant to two destructive alfalfa insects: alfalfa weevil {Hypera postica Gyllenhal} and potato leafhopper {Empoasca fabae (Harris)}. To aid us in elucidating the resistance mechanism, we studied the frequency and distribution of glandular hairs on two tetraploid (M. rugosa, M. scutellata) and two diploid (M. blancheana, M. disciformis) species. The four annual species have the upright capitate glandular hairs on all vegetative and floral parts except the cotyledons and flower petals. Hair density differed on plants grown in field and greenhouse, and plant parts and species reacted differentially. Density was not affected by ploidy level. Hair density was greatest on the peduncle followed by the stem, petiole and least on the pods and leaflets.

The diploids and tetraploids vary in the location of hairs on their leaves. Both have glandular hairs on leaflet margins, but on tetraploids, the hairs are distributed over the entire abaxial surface; on diploids, they are confined to the midrib area. When averaging the glandular hairs over all organs, M. scutellata (Turkey) has the greatest hair density followed by M. scutellata (Australia), M. scutellata (Argentina), M. disciformis, M. blancheana and M. rugosa.

Pollen tubes growth in the style of Medicago species was observed by use of fluorescence microscopy. The tissues of M. scutellata (annual) were softer than those of M. sativa L. (perennial). M. scutellata needed less time for a good softening at a specified normality of NaOH than did M. sativa so as to allow easy penetration of the dye and for good viewing of the pollen tubes in the style.

In the attempted interspecific cross between M. sativa and M. scutellata some pollen tubes entered into the ovule was observed. Most pollen did not germinate; however a few did but most of the pollen tubes did not penetrate into the style. M. scutellata pollen has diameter ranging from 0.032 - 0.046 mm. and M. sativa pollen have the same size as that of M. scutellata. No seeds were produced from this attempted cross.