Studies of Interspecific Sterility Mechanism in Species of *Melilotus*[1]

By

O. A. OJOMO[2] *and* H. J. GORZ[3]

ABSTRACT

Cytological studies of *M. infesta* revealed normal meiosis, and 2n = 16. High percentage of aborted and misshapen ovules, indicative of non-fertilization, and a probable incidence of somatoplastic sterility, following effective pollination, appear to be some of the interspecific sterility mechanism in species o *Melilotus*.

INTRODUCTION

THE use of commonly grown sweetclover, *Melilotus alba* and *Melilotus officinalis*, for forage is limited because of its susceptibility to the sweetclover weevil, *Sitona cylindricolis* (Fahraeus). The only known source of resistance to the weevil in the genus *Melilotus* is found in a small, unproductive annual known as *Melilotus infesta*. The resistance has to be transferred to *M. alba* or *M. offinalis* before it can be put to any practical use.

Interspecific hybridization provides a suitable method of introducing specific characters from non-economic to economic species, thereby augmenting their agronomic values.

From root tip investigations, Fryer (1930) and Mendoza (1946) reported eight pairs of chromosomes in several species of *Melilotus*. Meiotic studies by Webster (1950) reveal normal meiosis, but Jaranowski (1961) observed considerable aberrant chromosome distributions at meiosis in hybrids of *M. messanensis* x *M. segitalis*.

Ghosh *et al.* (1962) studied pollen fertility and cytology in two species of *Melilotus*. In both species, they obtained about 98% normal pollen using the stainable procedure, and 50% fertile pollen using pollen germination method. Meiosis was observed to be normal, and expressed in the high pollen fertility that was observed.

Despite the fact that all species within the genus *Melilotus* have a uniform number of chromosomes, interspecific compatibility has been very low, and only limited results of successful hybridization have been reported. Kirk (1964) and Johnson (1942) obtained only aborted seeds in the many crosses that they made.

Greenshields (1964) carried out cytological investigations of several crosses in *Melilotus* and proposed that nutritional factors were responsible for embryo failure. Growth of embryos proceeded normally for a few days before deteriorations set in. Endosperm was absent or abnormally developed, and necrosis of the suspensor was observed. Shastry (1960) observed that endothelial proliferation and persistence of coenocytic endosperm were precursors of seed failure. He contended that coenocytic endosperm was unable to supply food to the developing embryos.

Cooper and Brink (1940), working on interspecific hybridization in *Nicotiana*, reported an aggressive growth of inner integument, and hyperplasia of nucellus. They contended that these factors caused occlusion of the endosperm, with the consequence of ovule abortion. They called this phenomenon somatoplastic sterility.

Brink and Copper (1947) after an extensive study of hybridization in several angiosperms stated that "endosperm, embryo and maternal tissues must all be accounted for in an interpretation of the mechanism of seed failure". The work reported in the following pages includes investigations of cytology and pollen viability of *M. infesta*, and comparative histology of embryos from selfed plants of *M. infesta*, as well as of hybrid embryos of *M. infesta* x *M. sulcata* at various stages of development.

MATERIALS AND METHOD

Two accessions of *M. infesta*, obtained through the courtesy of George Stevenson and Hugo Gross, Canada Department of Agriculture, Manitoba, as well as four accessions of each of *M. sulcata, M. segitalis, M. italica* and *M. messanensis* were used in the study.

All the seeds were scarified and planted in cartons filled with a mixture of sand and vermiculite. The mixture was then covered with a thin layer of silica sand treated with orthocide (50% captan) at 0.00011 gm/ml., in order to reduce damping off of the seedlings. The seeds were germinated in a growth chamber maintained at 80°F, 50% relative humidity and 24 hours photoperiod for three weeks before being transferred to the greenhouse where they were transplanted into a sand-soil mixture in 4 inch clay pots.

Seeds were also grown for 5-10 days on wet blotters in petri dishes and root tips were removed for mitotic observations. The roots tips were pretreated with monobromonaphthalene (one drop in 50 ml of water) before being hydrolysed in I N H C L at 60-62°C for ten minutes and transferred to leuco-basic fuchsin stain solution. The root tips were then gently squashed on a slide. With this treatment, the chromosomes were differentially stained and became fairly well seperated.

1 Extract from thesis presented for M.Sc. at University of Nebraska in January 1966.

2 Present address: Research Division, Ministry of Agriculture and Natural Resources, Ibadan.

3 Professor and Geneticist, University of Nebraska, Lincoln, Nebraska. United States of America.

For meiotic investigations, racemes still in the very early bud stage were removed and fixed in Carnoys solution or Newmans fixative for at least 24 hours. The flower buds were then dissected and young anthers were squashed in a drop of either 2% aceto-carmine or 2% solution of propiono-carmine. Slight warming over a low flame facilitated spreading and staining of the chromosomes.

Pollen viability was studied under different conditions of growth, namely, in the green house, growth chamber and in the field. Two methods were used in studying pollen viability in *Melilotus infesta*. The first method involved the conventional staining of pollen with aceto-carmine. The second method involved growing of pollen in a liquid growth medium composed of 20% sucrose solution to which 10 ppm of boron has been added (Layne *et al*). All pollen that occured within the field of view of the objective lense were counted and since only germinated pollen was considered normal and viable, the values of germinated pollen were recorded as percentages of total pollen counted.

Various interspecific combinations were attempted with each of the five species of *Melilotus* that were planted. Flowers still in the bud stage were emasculated by pulling off the petals with flat-tipped tweezers, and the anthers were removed by suction developed by a motor-driven vacuum pump. Pollination was carried out by transferring pollen from a desired source to the stigma of the emasculated flowers. In this process, a toothpick to which a small piece of sand paper had been glued was found very useful.

Records were kept of percentage of fruit and seed that set.

For histological studies, *M. infesta* (as maternal parent) was crossed with *M. sulcata* and pods were removed from 20 hours to 15 days after pollination. Also, pods were simultaneously removed from *M. infesta* that had been selfed at the same time that cross-pollination was being made. The pod were embedded in molten wax following the method by McCarty (1965). Sectioning was done with a hand microtome at a thickness of 10-15 u, and the sections were stained in Safranin 0 (1% safranin 0 crystals in 56% ethyl alcohol) for 15 minutes and then counterstained with fast green (1% fast green FCF in 100% ethyl alcohol) for 30-60 seconds. The sections were permanently mounted in Canada balsam; and observed under oil immersion.

RESULTS

Cytology and pollen viability

Chromosome counts of root tips from *M. infesta* indicated that the normal chromosome complement is 2n = 16. A few cases of possible supernumerary chromosomes were observed.

Spore mother cells were more difficult to examine; a major difficulty was that chromosomes tended to clump together. Eight bivalents were observed in most cases at diakinesis, and only one case of a cell showing a quadrivalent was observed throughout the study. Normal orientation of the eight bivalents was observed at metaphase I and no lagging of chromosomes was observed at anaphase I. Regular tetrads were observed at anaphase in almost all cases following telophase II and no micronuclei were present.

The percentage of stainable pollen observed was high in all cases studies (table 1). There was no statistically

Table 1. Average percentage of stainable pollen and the frequency distribution of percentage stainable pollen in plants of *M. infesta* under various conditions of experiment.

Location of plants	*Percentage stainable pollen*						
	Percentage of population within each class range					*Mean*	*Total plants used*
	0-20	21-40	41-60	61-80	81-100		
Growth chamber	—	—	—	—	100	97.00	14
Green house	—	—	—	—	100	96.54	26
Field	7.7	—	—	—	92.3	91.24	13

Table 2. Average percentage of viable pollen and frequency distribution of percentage of viable pollen under various conditions of experiment.

Location of plants	*Percentage of viable pollen*						
	Percentage of population with each class range					*Mean*	*Total plants used*
	0.20	21-40	41-60	61-80	81-100		
Growth chamber	—	18.2	54.5	27.3	—	52.01	11
Green house	40	40	10	10	—	26.80	10
Field	7.7	7.7	30.8	38.4	15.4	58.78	13

significant difference between the values obtained for the three conditions of the experiment. In all cases also, the percentage germinable pollen (table 2) was much lower than comparable values for stainable pollen. The percentage of germinable pollen that was observed would appear adequate to ensure fertilization, after pollination may have been carried out.

Interspecific hybridization

No seeds resulted from the various interspecific crosses made. Many pods had dropped off one week after pollination, and what remained eventually shrivelled. Thus very poor results were obtained in all cases.

The results of dissection of many fruits under the binocular miscroscope indicated that:-

(1) About 27.8% were without recognizable ovules.
(2) About 68% had brown misshapen and shrivelled ovules.
(3) Less than 4.2% of the fruits had well developed ovules.

Histology

Seed development in *M. infesta* following either self-pollination or cross-pollination with *M. sulcata* was similar for the first 2-3 days following pollination. Fertilization occured from 20-30 hours after pollination, and occured more frequently in self-pollinated flowers.

3-4 days after cross-pollination, the proembryo in cross-pollinated ovules were surrounded by endosperm cells, and the nucellus remained prominent and often became many-layered. In self-pollinated ovules, the nucellus was less prominent and appeared partially absorbed and the proembryo much bigger.

5-6 days after cross-pollination visible viable embryos were rare, although endosperm was found in many of the slides that were made. At six days, many of the ovules were empty or contained debris of disintegrated embryos and endosperm in their embryo sacs. The nucellus however, persisted as a large-celled tissue, only a few cells thick in some places. In contrast, in self-pollinated ovules, the embryos were large and had differentiated into embryonic cotyledon, hypocotyl and radicle. The nucellus was completely disorganized at this stage.

DISCUSSION

The cytological observations, (2n=16) made in *M. infesta* are consistent with observations made by Shastry (1960) and Webster (1950) in their cytogenetic analysis of diploid species of *Melilotus*. The high level of stainable pollen (94.9%), and the relatively adequate percentage of viable pollen (45.8%) obtained from the same pollen sample seem to rule out meiotic irregularities and pollen abortion as the major cause of interspecific incompatibility in *M. infesta*.

Comparisons of post-fertilization stages following self and cross-pollination emphasize the wide divergence in type of development observed despite the initial similarities at the early stages of development. Stages of development following self-pollination indicate the presence of single-celled nucellar layer which flattened into a thin and gradually shrinking layer of cells. This eventually becomes completely absorbed after 6 days following self-pollination. At this stage the embryo develops cotyledonary primodia.

In contrast, following hybridization the nucellus becomes a much distended layer of collumnar cells with dense protoplasm and centrally placed nuclei. At 6 days, endosperm and embryo have become disorganized and unrecognisable. Thus the breakdown of endosperm following cross-pollination coincided approximately with the abortion of the embryo and hypertrophy of the prominent, single layered nucellus. Cooper and Brink (1940), have indicated similar findings that "a disturbed developmental relationship between these genetically diverse components is responsible for failure in hybrid seeds". However, the sequence of these events was not clear in this study.

Somatoplastic sterility as precisely described by Brink and Cooper (1940), and reportedly observed by Shastry (1960) appears to be of minor significance as a major factor causing the embryo failure. While hypertrophy of nucellus was observed, aggressive growth resulting in a multilayered nucellus was not observed. However, it is possible that such observed hypertrophy of the nucellus may be a variation of Brink and Cooper's somatoplastic sterility concept.

SUMMARY

M. infesta, a small unproductive plant that possesses a high level of resistance to attack by sweetclover weevil was subjected to cytological analysis, pollen viability and cross compatibility studies. Cytological investigation revealed that somatic cells contained 16 chromosomes with the possible presence of supernumerary chromosomes in some cells. At meiosis, the observation of eight bivalents at prophase and metaphase I, and normal tetrads after meiosis II, indicate a relative absence of meiotic irregularities.

High percentages of "normal pollen" (94.9%) and of viable pollen (45.8%) were observed. These high values of normal pollen appear highly correlated with the observed normal meiosis.

Various interspecific crosses were made with plants of *M. infesta* and four other species of *Melilotus*. Results that were obtained indicate a high degree of incompatibility between them. High quantities of aborted and misshapen ovules, indicative of non-fertilization and a probable incidence of somatoplastic sterility following effective fertilization may be some of the interspecific sterility mechanism in the *Melilotus* species studied.

REFERENCES CITED

1. BRINK, R. A. and COOPER, D. C. (1947). The endosperm in seed development. *Bot. Rev.* 13; 423-477, 479-541.
2. COOPER, D. C. and BRINK, R. A. (1940). Somatoplastic sterility as a cause of seed failure after interspecific hybridization. *Genetics* 25: 593-617.
3. FRYER, J. R. (1930). Cytological studies in *Medicago*, *Melilotus* and *Trigonella*. *Can. J. Res.* 3: 3-48 (quoted by Webster 13).
4. GHOSH, P. N., COOPER, D. C. and SMITH, W. K. (1962). Pollen fertility in *Melilotus*. *Phyton* 18: 105-107.
5. GREENSHIELDS, J. E. R. (1964). Embryology of interspecific crosses in *Melilotus*. *Can. J. Bot.* 32: 447-465.
6. JARANOWSKI, J. K. (1961). Semi-sterility in the interspecific hybrid *Melilotus polonica* x *M. alba*. *Amer. J. Bot.* 48: 28-35.
7. JOHNSON, I. J. (1942). Gross-fertility relationship of Golden annual sweetclover with common species of *Melilotus*. *J. Amer. Soc. Agron.* 34: 259-262.
8. KIRKE, L. E. (1951). Abnormal seed development in sweetclover. *Sci. Agric.* 10: 221-227.
9. LAYNE, R. C. and HAGEDORN, D. J. (1964). Effect of boron and agar on germination of pea pollen in sucrose media. *Crop. Sci.* 4: 33-34.
10. McCARTY M. K. (1965). Private communication.
11. MENDOZA, A. B. (1946). A survey of self-fertility and cross-compatibility between species of *Melilotus*. *M.Sc. Thesis Iowa State University*, quoted from Shastry et al., 12).
12. SHASTRY, S. V. S., SMITH, W. K. and COOPER, D. C. (1960). Chromosome differentiation in seven species of *Melilotus*. *Amer. J. Bot.* 47: 515-521.
13. WEBSTER, G. T. (1950). Fertility relationships and meiosis of interspecific hybrids in *Melilotus*. *Agron. J.* 42: 315-322.

Agricultural Extension as a Profession

ROBERT C. CLARK
Department of Extension Education and Rural Sociology, University of Ife

ABSTRACT

The important and complex tasks associated with agricultural development in Nigeria require the knowledge and skills of very capable Agricultural Extension Service Officers. Are such Officers considered to be professional personnel? They can be so considered IF their performance is based on a formal and somewhat standardized training programme; if their work is performed according to accepted standards of practice; if they exchange information and experiences with each other; if there is continued research to add to their knowledge and such information is reported in the professional literature: and if such officers are guided by a code of professional ethics and possess a strong sense of public responsibility. Evidence presented in this article points to the fact that the Agricultural Extension Service in Nigeria is attaining new levels of professionalism.

INTRODUCTION

THE level of professional competency is increasing in all fields of endeavour. The extent of formal study, the types of practical experiences and the high levels of professional achievement required of persons engaged in agriculture are continuing to increase each year. Certainly the professional qualifications expected of the Agricultural Extension Officer as an educator is no exception.

In the years ahead agriculture in Nigeria and throughout the world will be called upon, to an increasing degree, to supply added foodstuffs for a rapidly growing population. The development of densely populated urban centres, the establishment of home industries for processing agricultural products and additional export trade will require that agriculture become more specialized, more diversified, more mechanized and much more efficient than is the case today. The fact that approximately 70 percent of the income of Nigerians is spent on food (Anthonio, 1964) would, in itself, emphasize the continued importance of agriculture as a field of work that offers many professional opportunities.

A more scientific approach to the field of agriculture will be required if it is to measure up to the demands being placed on it today and in the foreseeable future. Many of the important tasks which must be performed are tasks for professionals.

The purpose of this article is three fold. First, the writer endeavours to define a profession by identifying its basic criteria set forth in the relevant literature on this subject. Secondly, the ideal characteristics of an Agricultural Extension Officer in general, as well as in Nigeria, are discussed. Thirdly, an attempt is made to answer the question, "Is Agricultural Extension a profession?".