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# IDENTIFICATION AND EVALUATION OF NEW ORNITHOPUS L. GERMPLASM WITH SPECIAL REFERENCE TO SEED CHARACTERS

A thesis presented in partial fulfilment

of the requirement for the Degree of

Master of Agricultural Science

in Seed Technology

at Massey University

Palmerston North

New Zealand

SHIMIN FU

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# ABSTRACT

Serradella species (Ornithopus L.) are promising annual forage legumes used in many parts of the world, especially in sandy and infertile dryland areas. One hundred and seven accessions of serradella were described and evaluated in Palmerston North , New Zealand. Fifty accessions were part of a 1986 DSIR sponsored forage germplasm collection from Southwest Europe. The rest of the accessions were introduced and/or collected from Australia and New Zealand. The study concentrated on the morphological description and evaluation of agronomic characteristics of the serradella accessions, as well as seed characteristics, including seed development and breaking of hardseed.

Four species (O. sativus, O. compressus, O. perpusillus and O. pinnatus) and one subspecies (O. sativus subsp. isthmocarpus) of serradella were identified and described in terms of their morphological characteristics. Morphological characteristics varied less than agronomic characters in serradella. For most accessions seed multiplication was successful. There was a relationship between flowering date and days to maturity, with the days from appearance of the first flower to seed maturity (harvesting date) decreasing as first flower emergence was delayed. Within species some accessions ripened in significantly shorter periods than others. Some had significantly heavier 1000 segment weight than others. In species normally considered to have a characteristic of high hardseed levels, a wide range in percentage hardseed was found among accessions of yellow serradella and slender serradella. In a study of seed development, seed viability of two serradella cultivars Grasslands 'Koha' (O. sativus) and a 'hybrid' (O. sativus x O. compressus) reached a maximum at 32 days after peak flowering. There was no germination unless fresh seed was dried. Hard seed developed at the age of 16 days in the hybrid. Soaking O. compressus and O. pinnatus seed in concentrated sulphuric acid for 30 minutes was the optimum treatment for breaking hard seed without causing seed damage.

TO THE MEMORY OF DR. MARGOT B. FORDE

A RESPECTED FRIEND AND SUPERVISOR

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## APPENDICES

# INTRODUCTION

Plant exploration, together with notable contributions from plant breeding and selection, has contributed greatly to the use of legumes for pasture improvement ('t Mannetje et. al., 1980). This study is a contribution to the description and evaluation of some serradella species collected for pasture germplasm resources by Dr. M.B. Forde and Dr. H.S. Easton, DSIR Grasslands, Palmerston North, New Zealand in 1986. During their trip to SW Europe, 50 accessions of serradella (Ornithopus) were collected. These had not been grown and assessed for agronomic characteristics until this study was started in 1990.

The description of accessions and the recording of the information in databases are aspects of genetic resources work which have progressed slowly. The large number of samples now conserved in genebanks makes their comprehensive description and evaluation a formidable task (Williams, 1989).

The controlled use of 'wild' plant germplasm is largely dependent upon accurate description and taxonomic identification, followed by comprehensive evaluations by competent authorities. The thoroughness of such evaluation depends upon the number of accessions under observation at a given time, the diversity of genera or species, and the availability of specialists. Transferring a plant from its original habitat to a different location and environment often results in phenotypic differences, and in many cases unconscious selection results in genetic change. Thus descriptive records, from the time of original discovery and throughout the evaluation period, become highly important. They should be prepared for long-term reference, and assembled methodically for computer adaptation (Hyland, 1970).

Ornithopus is a small leguminous genus of six species and subspecies. Five species and subspecies are found in Mediterranean regions of Western Europe and North Africa, and one

in South America. The species are soft pubescent annual herbs with finely divided leaves, small pink or yellow flowers and indehiscent pods which break into one-seeded segments. The common name for these plants is " serradella ", in reference to the serrate leaflets. Adaptation to sandy soils appears to be a common feature throughout the genus (Gladstones et. al., 1964).

The species are all highly palatable and are used for forage in their areas of origin, and also in Eastern Europe, United States of America, Australia and New Zealand. The most commonly used species are yellow serradella (O. compressus), pink or French serradella (O. sativus) and slender serradella (O. pinnatus).

The seed characteristics of these species are of great importance for their agronomic usage. For successful reseeding in some environments it is essential that hard seed builds up in the soil, but if all the seed is hard then there is not enough immediate germination. O. sativus lacks hard seed, while O. compressus may have too much hard seed. Embryo dormancy can also be a problem.

The objectives of this study were:

(1) to identify and describe the accessions and harvest seed for further study;

(2) to study the seed characteristics of each species and investigate the variation between accessions with respect to the potential for genetic improvement in agronomically important characteristics such as hard-seededness, dormancy and hullability.

(3) to investigate methods for dehulling and breaking hard seed.

In this study, one hundred and seven accessions (both collected and introduced) were described for morphological and agronomic characteristics and tested for seed multiplication. Some were also tested for seed characteristics, seed development and a method for breaking

hard seed. The study was divided into three parts. The first experiment concentrated on the description and multiplication of all accessions. Seed development of two serradella cultivars was recorded in the second experiment, while in the third, the use of concentrated sulphuric acid to break hard seed was tested in two serradella species, O. compressus and O. pinnatus, both of which have a high percentage of hardseed.

# CHAPTER 1

# LITERATURE REVIEW

## 1.1. Serradella (Ornithopus sp): Origin, Characteristics and Classification

### 1.1.1. Origin

The species in the genus Ornithopus occur mainly in the countries surrounding the Mediterranean, along the Atlantic seaboard of western Europe, also western Asia and tropical Africa. One species occurs in southern South America.

The common name, serradella may be derived from the Portuguese 'serradilla' which is a diminutive of 'serrado', meaning serrate (Gladstones et. al., 1964). Also, it has been suggested that the name serradella was taken from one of the villages where it grows i.e. the village of Serradilla del Arroyo, south-east of Coudad Rodrigo in Salamanca Province of Spain. Moreover, it may have come from a Spanish word 'serradello' which means 'little saw', and describes the saw-like leaf of serradella (Gladstones et. al., 1977).

The generic name Ornithopus is likewise derived from the Greek ornis, a bird, and pous, a foot. It should not be confused with Lotus corniculatus L., the so-called birdsfoot trefoil (Gladstones et. al., 1964). Both names refer to the clusters of pods which resemble a bird's foot.

### 1.1.2. Characteristics of the genus and species

### 1.1.2.1. Botanical characteristics

The genus is described in 'Flora Europaea' (1968) as follows:
Annual. Leaves imparipinnate; stipules small, free, linear. Flowers in axillary heads. Calyx tubular or campanulate, with 5 equal teeth; keel obtuse; stamens diadelphous. Legume lomentose, terete or compressed, usually constricted between segments, strongly reticulate.

Bentham and Hooker (1865) and Allen et. al. (1981) gave a description as: Herbs, annual, stems much-branched, delicate, slender, spreading or erect, unarmed, some pubescent. Leaves imparipinnate; leaflets small, 3-18 pairs; stipels absent; stipules small, free, linear. Flowers minute, yellow, white, or pink in small heads or umbels on long, axillary peduncles, sometimes subtended by a floral leaf; bracts and bracteoles small; calyx tubular or campanulate; lobes 5, equal, or the upper 2 connate above; standard obovate or suborbicular; wings oblong; keel obtuse, nearly straight, shorter than the wings; stamens 10, diadelphous; alternate filaments usually dilated above; anthers uniform; ovary sessile, many-ovuled; style curved, not bearded; stigma capitate. Pod usually curved, cylindric or flattened, beaked, tomentose, with constrictions between the 3-8 segments, each segment reticulate, ovate or oblong, 1-seeded, indehiscent; seeds transversely oblong, ovate, or subglobose.

At present, six species are contained in the genus. Five species are distributed in Europe, the Mediterranean region, western Asia and the Atlantic Islands, and one species is in southern South America. All of them are 2n = 14.

### 1.1.2.2. Species and distribution

In general, the main way to classify the six species (O. sativus, O. isthmocarpus, O. compressus, O. pinnatus, O. perpusillus, O. micranthus) in the genus is by classical methods e.g distinguishing different species by morphological characteristics. Five species have been described in 'Flora Europaea'. To distinguish them, the key to the species is shown below:

1 Heads ebracteate or with minute scarious bracts **4. pinnatus**

1 Heads subtended by a pinnate leafy bract

2 Corolla yellow; legume not or only slightly contracted between the segments **1. compressus**

2 Corolla pink or white; legume strongly contracted between the segments

3 Corolla 6 mm or more; bracts shorter than the flowers, usually about equalling the calyx **2. sativus**

3 Corolla not more than 5 mm; bracts much longer than the flowers **3. perpusillus**

1. O. compressus L., pubescent, stems 10-50 cm. Leaflets 7-18 pairs, oblong, elliptical or oblong-lanceolate. Heads 3- to 5-flowered; bracts with 7-9 leaflets. Calyx-teeth at least half as long as the tube; corolla 5-8 mm, yellow. Legume 20-50 mm, curved, more or less compressed, not or only slightly contracted between the segments; segments 5-8, oblong; beak 7 mm or more, curved.

2. O. sativus Brot., pubescent, stems 20-70 cm. Leaflets 9-18 pairs, lanceolate or elliptical to ovate. Heads 2- to 5-flowered; bracts with 5-9 leaflets, shorter than the flowers. Calyx-teeth slightly shorter than to about equalling the tube; corolla 6-9 mm, white or pink. Legume 12-40 x 2-2.5 mm, compressed, contracted between the segments; segments 3-7, elliptic- oblong.

(a) Subsp. sativus: Legume 12-25 mm, straight; beak usually not more than 5 mm, straight, sometimes hooked at the tip.

(b) Subsp. isthmocarpus: Legume 20-40 mm, curved, usually with a long, narrow, cylindrical constriction between the segments; beak 10 mm or more, curved.

3. O. perpusillus L., pubescent, stems up to 30 cm. Leaflets 7-13 pairs, elliptical or oblong. Heads 3- to 8-flowered; bracts with 5-9 leaflets, longer than the flowers. Calyx-teeth not more than half as long as tube; corolla 3-5 mm, white or pink. Legume 10-18(-25) x 1.5-2 mm,

straight, compressed, contracted between the segments; segments 4-9, elliptic-oblong, beak not more than 3 mm, straight, often hooked at the tip.

4. O. pinnatus (Miller) Druce, glabrous or sparsely pubescent, 10-50 cm. Leaflets 3-7 pairs, linear to oblanceolate. Heads 1- to 5-flowered, ebracteate or with minute scarious bracts. Calyx-teeth not more than half as long as tube; corolla 6-8 mm, yellow. Legume 20-35 mm, curved, terete, not contracted between the segments; segments (6)8-12, cylindrical; beak not more than 5 mm.

( adapted from "Flora Europeae" (1968) )

An indigenous South American species, O. micranthus (Benth.) Ar., is a cattle forage of minor importance in Argentina (Allen et. al., 1981), but the species does not appear to have been described.

Gladstones et. al. (1977) listed these species and their distributions as given below (Table 1-1).

**Table 1-1.** Species of serradella and their distribution

| Botanical name | Common name | Distributions |
|---|---|---|
| Ornithopus sativus Brot. | French serradella | Known only in cultivation, mainly western and northern Europe. |
| O. isthmocarpus Cosson (or O. sativus ssp. isthmocarpus Cosson) | Moroccan serradella | Coastal Morocco, coastal S. Spain and Portugal. |
| O. compressus L. | Yellow serradella | Pan-Mediterranean to 1500 m. Atlantic coast from Central Morocco to Isle of Wight. Madeira. Canary |

| | | |
|---|---|---|
| | | Is. Cultivated in Spain, W. Australia. |
| O. pinnatus (Mill.) Druce | Slender serradella | Pan-Mediterranean to 600 m.Atlantic Coast from Central Morocco to Scilly Is. Acid, sandy soils. |
| O. perpusillus L. | Common birdsfoot | Pan-European, common on lowland sandy and stony soils. Morocco (rare). |
| O. micranthus (Benth.) Ar. | | Southern South America, Argentina |

### 1.1.2.3. Breeding system

The species in the genus are regarded as self-pollinated species. Pollen germination occurs prior to anthesis, which is known as precocious bud-pollination. Also, it is thought that cross-pollination occurs frequently between O. sativus and O. perpusillus.

Wojciechowska (1972) investigated pollination and fertilization in Ornithopus spp. using three species (O. sativus Brot., O. perpusillus L. and O. compressus L.). Eight stages of bud and flower development were set apart for observation:

I - bud green, corolla invisible,

II - petals hardly visible among the sepals,

III - petals and tips of sepals on the same level,

IV - petals slightly outgrowing the sepals,

V - petals notably elongated, notably outgrowing the sepals,

VI - petals fully developed, yet unfolded,

VII - flower fully open,

VIII - flower withered, petals wrinkled and stained.

From observations of maturation of anthers and of pollen grains in situ as well as results from germinating pollen grains in vitro (Wojciechowska, 1972) it was clear that in a developing flower bud there exist conditions which favour self-pollination. The onset of anther bursting occurred during stage IV (petals slightly outgrowing the sepals), while at stage V (petals notably elongated, and growing above the sepals) pollination was a mass event. Stigmata were surrounded at this period by anthers shedding pollen directly onto this organ, and thus providing for a successful pollination. At the time of pollen shedding, the stigma was mature and pollen grains immediately started to germinate. The onset of germination took place at stage IV (petals somewhat outgrowing the sepals). The greatest number of germinating pollen grains was noticed prior to opening into bloom (stage VI) and at the period of peak bloom (VII). Before flower opening a large number of pollen grains had germinated, and pollen tubes had penetrated into the stylar canal. Some fertilization and development of the proembryos had already occurred. Fertilization of ovules in the flower bud was observed in the first place in O. compressus.

Cytological-morphological observations showed that in a developing flower bud there exist conditions favourable for selfpollination and it can be assumed that processes of autogamy are in preponderance. Knuth (1898) noted that owing to the position of the stigma and stamens in respect of one another, spontaneous self-pollination was inevitable in O. sativus and O. perpusillus. Similar conclusions were arrived at by Fruwirth (1921), Heuser and Pfrang (1933) and Klinkowski (1942). Likewise breeding experiments provide supporting evidence. Serradella planted under isolation exhibited a satisfactory pod and seed setting, without symptoms of inbreeding depression (Wojciechowska, 1972).

Published data justify the statement that all tested species and forms of serradella belong to basically self-pollinating plants. Assumptions that some cross-pollination may occur in serradella partly stem from observations of a mass visiting by insect pollinators

(Wojciechowska, 1972). Wojciechowska nevertheless suggested that for breeding purposes serradella can be regarded as a self-pollinating species. However, closer genetic investigations require isolation of individual plants.

#### 1.1.2.4. Nodulation

Rhizobia strains from serradella nodules were acknowledged as strains of Rhizobium lupini about the turn of the century (Maassen and Miller 1907, Simon 1907; Allen et. al., 1981). It had become obvious that serradella growth and establishment was improved on sites where lupines had previously grown. Furthermore, Jensen and his co-workers established that the symbiotic range of serradella-lupin rhizobia is much broader than it was originally conceived to be (Allen et. al., 1981).

### 1.1.3. Classification methods

#### 1.1.3.1. General

The recent history of the development of taxonomy is very largely a reflection of the development of techniques which have a taxonomic application. These techniques have seldom been developed by taxonomists but by scientists working in related biological fields, and more recently by workers in quite diverse fields. In the 1940's the newer techniques applied were those of cytology, ecology, genetics and combinations of these such as genecology and cytogenetics (Heywood, 1968a).

The picture since then is dramatically different, largely because of the application of new methods, in particular chemical techniques such as chromatography and electrophoresis. The rapid and efficient screening of chemical compounds and consequently their quick identification, led to the field known as biochemical systematics which has itself evolved rapidly during the past few years; computer technology and electronic data processing have

also had a major impact on systematics, particularly numerical taxonomy or taximetrics (Heywood, 1968b).

Cytology, the study of the cell, or more properly the cell nucleus, has been an important tool in the elucidation of evolutionary and biosystematic problems. The characteristics of chromosomes and their behaviour during cell division and gamete formation have provided a great deal of relevant information. The importance of chromosomes is twofold: first, their number, behaviour and characteristics can be used in the classification of species; and second, chromosomes can give an insight into genetic phenomena and the evolutionary processes that lead to the formation of species. Like all characteristics of an organism, chromosomes and the mechanisms of cell division and of gamete formation are subject to natural selection. Therefore, they will vary from species to species as a result of different evolutionary histories (Solbrig, 1970).

Chemical systematics, that is the use of chemical data of all sorts in systematic investigations, has blossomed and is now a recognized and very active area of systematics. The study of all chemical reactions taking place in a cell throughout the life of an organism is an enormous task. The major plant constituents have been isolated and their chemical structure identified. Many have been synthesized in the laboratory, and in many cases their biosynthesis has also been learned (Solbrig, 1970).

Computers are being increasingly employed in the development of quantitative methods of classification; the role of the computers is to work out the quantitative comparisons between organisms in respect of a large number of characters simultaneously. Numerical taxonomy is based on phenetic evidence, that is to say on the similarities shown by observed and recorded characters of taxa, not on phylogenetic probabilities (Heywood, 1968a).

### 1.1.3.2. Electrophoresis

With the development of agriculture, more and more crop and pasture cultivars are being bred by plant breeders. Distinguishing between those cultivars which are very close to each other within the same species has become increasingly important to plant breeders, for plant variety rights, and for seed certification authorities.

Usually, the identification methods used are based on morphological characteristics to identify different plant species and cultivars. However they are not easily mastered because there may be considerable variation when plants are grown outside, and identification accuracy is likely to be affected by this factor. Because of this, small variations of morphological characters often result in disputes. For example, it is quite common to treat the same species, subspecies or variety at different taxonomic levels, which is the problem botanists and taxonomists have been facing. As a complement, the application of a new technique, such as gel electrophoresis may be useful for species or variety identification.

In recent years, electrophoresis has been widely used in crop and pasture cultivar identification. The importance of methods of gel electrophoresis for the classification and identification of cultivars of agricultural and horticultural species is now firmly established (for reviews see Wrigley et. al., 1982; Cooke, 1984, 1986, 1988). The electrophoretic analysis of proteins and/or enzymes has been very widely applied, ranging from the breeding of new cultivars, through seed testing, production and certification, to the selling of the harvested seed and its commercial processing (Cooke, 1988, 1989).

In 1986, after a two year programme of collaborative interlaboratory testing, the International Seed Testing Association (ISTA) adopted a standard reference method of polyacrylamide gel electrophoresis (PAGE) for the identification of varieties of wheat and barley into its International Rules (Cooper, 1987; Cooke, 1989). The method involves separation of the alcohol-soluble seed storage proteins (gliadins from wheat, hordeins from

barley) by PAGE at pH 3.2 using 10% acrylamide gels and a glycine/acetic acid buffer. Other techniques, such as PAGE in the presence of sodium dodecyl sulphate (SDS-PAGE) and isoelectric focusing (IEF), have also been successfully utilized for identification of wheat and barley varieties (Wrigley et. al., 1982; Cooke, 1984, Smith & Payne, 1984; Cooke, 1989).

It has already been noted that there is a wide range of methodology available for the electrophoretic discrimination of genotypes in cereals. Because the various types of electrophoresis (acid PAGE, SDS-PAGE,IEF) depend on different physical properties of proteins to bring about their separation, it is often possible to use methods sequentially and hence improve the degree of resolution between genotypes (Cooke, 1989).

At present, one of the most commonly used methods for the identification of crop and pasture cultivars is SDS-PAGE. The basic theory of SDS-PAGE is that seeds of different and closely related cultivars often show considerable polymorphism with respect to their storage proteins. When SDS is present, it makes the charge density of each protein monomer become equal so that separation completely depends upon the molecular weight of the proteins. The differences in banding pattern produced by protein electrophoresis are used as the basis for identifying cultivars. Usually, the operation of SDS-PAGE is divided into four steps: a) protein extraction, b) gel preparation, c) running the samples and d) staining (retrieving the gels and visualising the different protein bands) (Coolbear, 1989).

SDS-PAGE has been successfully used for distinguishing some species and cultivars in the genera Lolium, Festuca, Dactylis, Bromus, Ceratochloa, Pisum, Phaseolus, Glycine, Arachis, Trifolium, Lotus, Ornithopus and Vicia (Gardiner et. al., 1988; Cooke, 1989).

The usefulness of seed protein profiles to identify species and cultivars, as well as the information provided about genetic relationships, makes SDS-PAGE of seed proteins a valuable tool for plant breeders and certification authorities, and also in genetic resource management (Forde et. al., 1986, 1988; Gardiner et. al., 1988).

As most cultivars of pasture legumes produce a characteristic banding profile of seed proteins, the identity of a seed line may be checked rapidly without the necessity for time-consuming field evaluations of spaced plants. Seed protein analysis by SDS-PAGE has been successfully used a number of times by plant breeders to check pedigrees, and also to test for genetic shift after seed multiplication or re-isolation. In addition, it is of particular value for identifying hybrid and back-cross progenies (Gardiner & Forde, 1988).

Electrophoresis represents a highly discriminating means of differentiating between genotypes, which could clearly be of some importance for DUS testing purposes (Cooke, 1989). In genetic resource management, seed protein profiles have proved an ideal tool for checking species identifications and identifying duplicate accessions (Gardiner & Forde, 1988).

## 1.2. The Role of Serradella in Agriculture

### 1.2.1. Climatic adaptation

All species of serradella are annual pasture plants with good palatability and special adaptation to sandy and acid soils. Gladstones and McKeown (1977) summarised their habitats and naturaldistribution:

Ornithopus sativus (French serradella) is cultivated in France, also on the poor sandy soils of the Baltic coastal region in northern Europe, extending into western Russia. Here it provides high quality forage on soils too poor for most other forage plants. This species has an erect growth habit and fully permeable (soft) seeds.

O. compressus (Yellow serradella) is by far the commonest and most widespread of the species in the wild state. It occurs in all regions bordering the Mediterranean which have non-calcareous soils and an annual rainfall exceeding about 400 mm, extending northwards along

the Atlantic coast as far as the Channel Islands. In Morocco, Spain and southern Italy it grows from sea level to altitudes of about 1500 metres, on acid sands to neutral loams. It covers almost all soil types on which subterranean clover is found, plus a wide range of sandy and gritty soils from which subterranean clover is absent.

O. pinnatus (Slender serradella) occurs over much of the same geographical range as yellow serradella, but is confined to altitudes below about 600 m, and almost exclusively to acid sands of very low fertility.

O. isthmocarpus (Moroccan serradella) is confined strictly to the sandy coastal lowlands of western Morocco and of southern Spain and Portugal; a distribution closely resembling that of the sandplain lupin Lupinus cosentinii.

O. perpusillus (Common birdsfoot) is mainly distributed in central and north-west Europe, but is occasionally found as far south as Morocco. It is a fairly common volunteer on poor soils through much of its range.

It is reported that there is an indigenous South American species, O. micranthus in Argentina, but little information about this species is available.

### 1.2.2. Role in agricultural systems

Ornithopus species, especially O. compressus L. and O. sativus Brot., are cultivated in various parts of the world for grazing, soil cover and improvement, and for hay and silage. These two species have become particularly popular in Western Australia for sandy soils where Trifolium subterraneum has not flourished (Parker and Oakley, 1963; Allen et. al., 1981).

Gladstones et. al. (1964) summarized and mentioned some experimental situation of O.

sativus in different countries. That was, in more recent years, O. sativus has been grown experimentally in many countries and has met with particular success in South Africa, where it is considered an excellent forage legume in both the winter rainfall area of the South-West Cape Province (Sim, 1959) and the South East Cape region (Shone, 1962). It has also been recommended for high altitudes in the tropics, in the Congo (Whyte et. al., 1953) and Kenya (Strange, 1955).

Serradella has shown promise as a winter legume for forage and soil improvement in sandy soils in the southeastern United States (Allen et. al., 1981).

### 1.2.2.1. Soil cover and improvement

One of the most valuable characteristics of serradella is its well established ability to grow on deep sandy soils of low fertility. One of the reasons for the success of serradella on deep sands undoubtedly lies in its deep root system which enables it to exploit nutrients and moisture reserves in the deeper soil layer and to continue growing longer into the spring than shallow-rooted species such as subterranean clover under the same conditions (Gladstones et. al., 1964).

Ozanne et al.(1965) carried out an experiment in root distribution in a deep sand with 12 temperate annual pasture species. At day 126 in the field experiment, root distributions of the 12 species were as shown in Figure 1-1.

Some roots of all species penetrated to a depth of 155 cm. In silver grass and the two strains of subterranean clover, however, these roots represented only a very small proportion of the total. Thus Yarloop subterranean clover had only 0.1% of its roots deeper than 80 cm, silver grass had 0.2%, and Mt. Barker subterranean clover 1.0%. By contrast, the relatively deep-rooted species serradella, cape-weed, and erodium each had more than 10% of their roots in the 80-155 cm layer, and lupins had 7.8% of their roots between 80 and 155 cm.

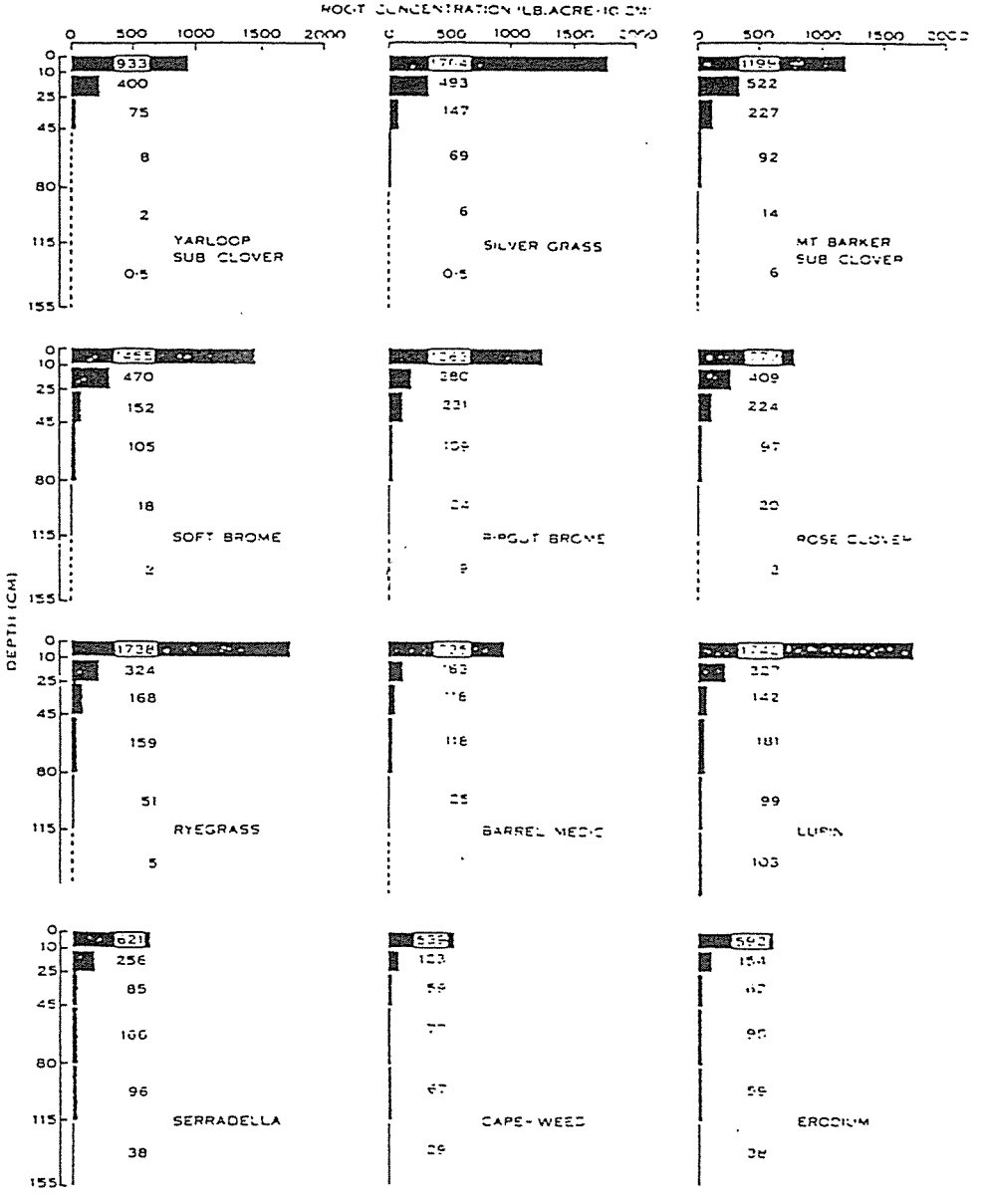



Fig.1–1 Relationship between root concentration (lb acre-10 cm) and depth (cm) for 12 species grown for 126 days in the field. (Broken lines indicate less than 3 lb acre-10 cm.) Weight of roots (lb acre) for each depth interval are also shown.

Serradella can withstand a pH of about 4.7, but becomes more vigorous at a pH of up to about 5.5. It does well on sandy peats (Halliwell, 1960).

Because of its deep root system, fast growing and prostrate habit, serradella has good potential as a soil cover.

There is some evidence that serradella is very efficient as a supplier of nitrogen (Halliwell 1960). Features such as its high adaptability to infertile, deep sandy soil, a broader symbiotic range of rhizobia, and not suffering from the problem of second-year-mortality are closely related to a nitrogen supplier.

Australian Department of Agriculture trials have shown that serradella needs slightly less copper, zinc and molybdenum than subterranean clover. Comparative analyses of serradella and other pasture legumes grown on the same soil (Table 1-2) suggest that this is due to serradella's greater ability to take up these elements from the soil (Gladstones et. al., 1977).

Table 1-2 Compositions (whole tops) of different pasture legumes grown under the same conditions at Gidgegannup, Western Australia, and sampled a little before maturity (October 14-18).

| | Crude protein per cent[1] | Calcium per cent[2] | Magnesium per cent[3] | Phosphorus per cent[3] | Manganese parts per million[3] | Zinc parts per million[4] | Copper parts per million[5] | Cobalt parts per million[3] | Selenium parts per million[6] |
|---|---|---|---|---|---|---|---|---|---|
| Ornithopus sativus (French serradella) .... .... | 20·1 | 1·22 | 0·34 | 0·27 | 107 | 62 | 8·0 | 0·106 | 0·012 |
| O. compressus (yellow serradella) cv. Pitman .. | 20·1 | 1·31 | 0·38 | 0·25 | 106 | 58 | 7·5 | 0·087 | 0·030 |
| Trifolium subterraneum (sub clover) cv. Bacchus Marsh | 15·0 | 1·21 | 0·35 | 0·15 | 46 | 31 | 5·7 | 0·088 | 0·009 |
| Trifolium subterraneum (sub clover) cv. Clare .... | 12·9 | 0·88 | 0·24 | 0·13 | 30 | 24 | 5·4 | 0·082 | 0·017 |
| Trifolium hirtum (rose clover) cv. Kondinin .. | 16·0 | 1·23 | 0·30 | 0·14 | 34 | 33 | 5·4 | 0·056 | 0·011 |
| Medicago truncatula (barrel medic) cv. Hannaford | 14·4 | 1·06 | 0·33 | 0·14 | 28 | 20 | 5·3 | 0·061 | 0·018 |

[1] After Gladstones, J. S. and Loneragan, J. F. (1975)– Aust. J. Agric. Res. 26: 113-26.
[2] After Loneragan, J. F., Gladstones, J. S. and Simmons, W. J. (1968)—Aust. J. Agric. Res. 19: 353-64.
[3] Loneragan, J. F., Gladstones, J. S. and Simmons, W. J.—unpublished data.
[4] After Gladstones, J. S. and Loneragan, J. F. (1967)—Aust. J. Agric. Res. 18: 427-46.
[5] After Gladstones, J. S., Loneragan, J. F. and Simmons, W. J. (1975) – Aust. J. Agric. Res. 26: 103-12.
[6] Loneragan, J. F., Gladstones, J. S. and Buckley, R.– unpublished data.

Capacity to take up nutrients from depth and recycle them to the surface could be expected to have beneficial effects on the total nutrient economy of the soil, especially for soluble mineral nutrients such as potassium, which are readily leached beyond the reach of shallow-rooted plants in sandy soils, and so become permanently lost (Gladstones et. al., 1977).

Serradella is a primary fertility-building species, but it also gives excellent grazing returns of protein and minerals at a critical period of the year (Halliwell, 1960).

### 1.2.2.2. Grazing value

According to all the evidence available, serradella has high value as a stock feed. Overseas publications refer to O. sativus as being very suitable for hay or silage and make no reference to any form of toxicity. Both O . sativus and O. compressus have a leafy growth habit and are relatively free from woody stems. Western Australian serradella is particularly satisfactory in these respects. The pods, which form above ground, are small and relatively non-woody, and should constitute a readily available source of protein for stock during the summer (Gladstones et. al., 1964).

Serradella has an extremely high feed value, and contains no oestrogens. It maintains its protein and mineral content during maturation to a greater extent than most other pasture plants. Table 1-2 shows the contents, a little before maturity, of crude protein and of various major and trace elements in the two common serradella species, as compared with other pasture legumes of similar maturity, grown with the same fertiliser treatment on a light gravelly soil at Gidgegannup, Western Australia. (For the five trace elements, the figures are means of the three application rates of each element.) The data show serradella to be particularly rich in protein, phosphorus, manganese, zinc and copper (Gladstones et. al., 1977).

In persistency under grazing, O. compressus is better than O. sativus, which is related in part to its high hard seed content (hard seeds remaining in the soil germinate next growing season). However there are also differences in growth habit which could be important. O. compressus has a dense, spreading growth habit and even in thick stands bears at least a proportion of its flowers and pods close to the ground, where they have a good chance of escaping grazing under conditions of rapid growth in spring. On the other hand O. sativus grows more erect and normally bears its flowers and pods further up the stems, suggesting that it would be more vulnerable to grazing (Gladstones et. al., 1964).

The outstanding feature of this legume in Northland, New Zealand is that it is able to grow

well on sandy coastal soils during late winter and early spring, the seasons of shortages in grassland production (Halliwell, 1960).

### 1.2.2.3. Dry matter and seed production potential

Because of its characteristics, serradella shows a high dried herbage production potential where the climate is suitable for its growth (Fig. 1-2). Bolland (1985) reported that fertilizer nitrogen applied regularly to the serradella sward markedly improved herbage yield in the winter, but not in the warmer spring. In his experiment, one of his objectives was to know whether serradella grew poorly during the winter, and if so, why; compared with two subterranean clover cultivars, what was the herbage productivity of three serradella species

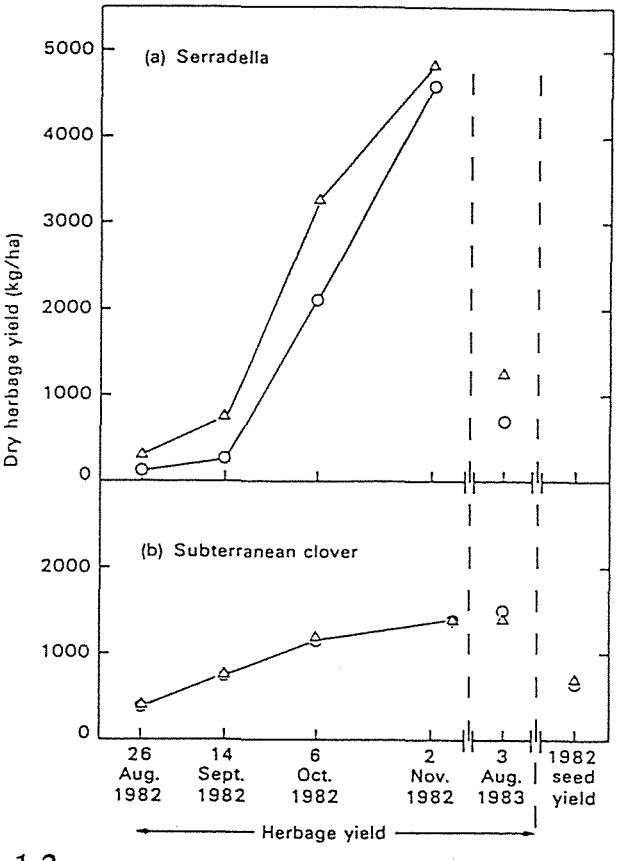



Fig. 1-2. Herbage and seed yields at each harvest, for the plus and minus fertilizer nitrogen treatments, in the main experiment. The data are means of the cobalt treatments: o, no-nitrogen treatments; △, plus-nitrogen treatments.

in the absence and presence of regular applications of nitrogen in the winter and spring, and whether low temperatures in winter limited the rate of symbiotic nitrogen fixation. The results showed that regular applications of nitrogen fertilizer had no effect on the herbage or seed yield of subterranean clover, but had a marked effect on the dry herbage yields of serradella in winter (Table. 1-3). There were no effects of nitrogen treatments on seed yield. He concluded that low temperatures limit the rate of symbiotic nitrogen fixation in the root nodules of serradella, which in turn limits herbage yield in winter.

Table 1-3. Herbage and seed yields (kg/ha, mean±s.d.) measured in the ancillary experiment during 1983 −N, no-nitrogen treatments; +N, plus-nitrogen treatments.

| | Dried herbage yield | | | | Seed yield | |
|---|---|---|---|---|---|---|
| | 2 Aug. 1983 | | 26 Sept. 1983 | | 2 Feb. 1984 | |
| | −N | +N | −N | +N | −N | +N |
| *O. compressus* cv. Pitman | 52±16 | 148±14 | 2044±118 | 2015±110 | 471±31 | 456±115 |
| *O. pinnatus* GT 045 | 9±4 | 71±26 | 1706±124 | 1686±146 | 395±41 | 379±56 |
| *O. perpusillus* GM 034 | 52±10 | 168±36 | 1937±98 | 1913±102 | 379±51 | 375±45 |
| *T. subterraneum* cv. Daliak | 159±16 | 175±58 | 1739±80 | 1600±88 | 690±117 | 589±165 |
| *T. subterraneum* cv. Seaton Park | 205±24 | 243±50 | 1753±112 | 1763±124 | 494±42 | 546±77 |

(adapted from Bolland, 1985)

Another trial performed by Bolland (1986) indicated a relationship between seeding rate and maturity grading. Seed yields increased with increasing seeding rate and decreased with increasing maturity grading, the decrease being more marked as the seeding rate increased (Fig. 1-3, 1-4).

Also, his experiment indicated a high seed production potential in some strains and cultivars of yellow serradella, and some other species of serradella (Fig. 1-3, 1-4).

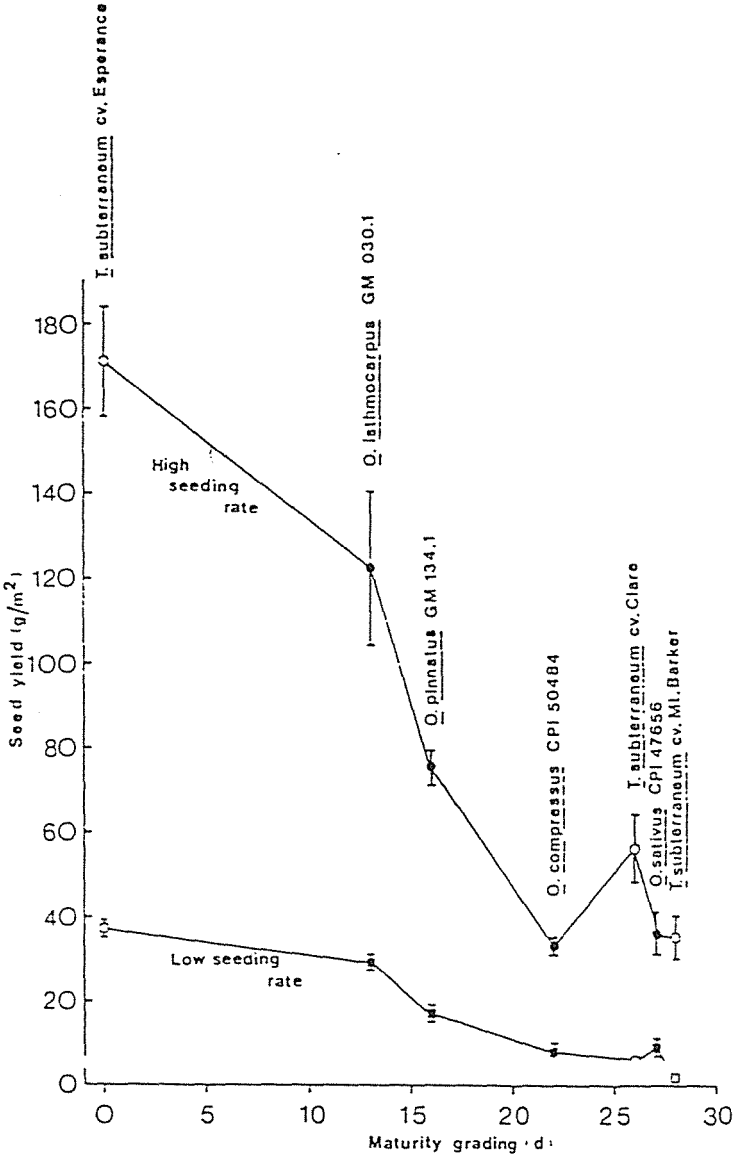



Fig. 1-3 Relationship between seed yield and maturity grading (MG) for strains and cultivars of four serradella species and subterranean clover measured in 1982 for seed sown at two seeding rates on 2nd June 1982 (Experiment 2). MG was calculated relative to the earliest flowering legume in the experiment, cv. Esperance, for which MG=0. Vertical bars are standard errors of the mean for each point. The seed weight differs for the various species studied, and seeding rates were chosen (see Table 2) to give similar plant densities for all the species, strains and cultivars for each of the two seeding rates.

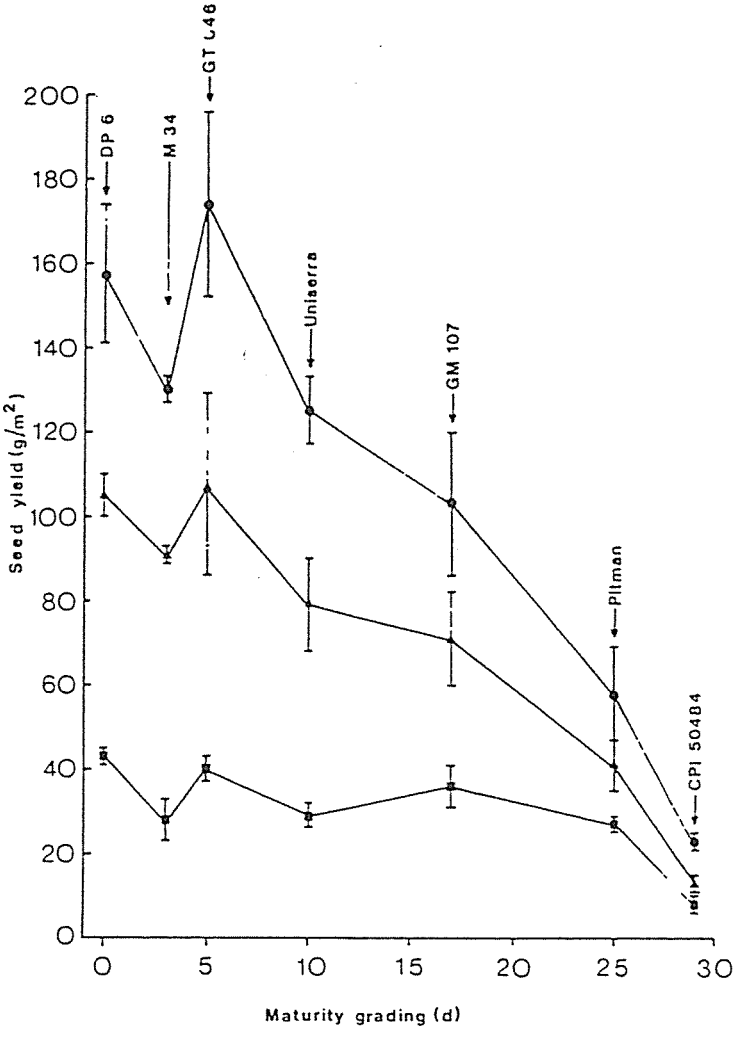



Fig. 1-4. Relationship between seed yield and maturity grading (MG) measured in 1982 for strains and cultivars of *O. compressus* for seed sown at 2, 20 and 80 kg/ha on 2nd June 1982 (Experiment 1). MG was calculated relative to the earliest flowering strain in the experiment, DP6, for which MG=0. Vertical bars are standard errors of the mean for each point.

### 1.2.2.4. Agronomic requirements

Most successful stands of serradella in Western Australia are in medium to high rainfall districts, with an annual rainfall of about 20 inches (about 500 mm) or more (Gladstones et. al., 1964). Serradella is considered to be the only annual pasture legume to persist on well-drained, deep (greater than 50 cm) sandy, neutral to slightly acid soils (Gladstones et. al., 1964, 1977, and Bolland, 1985), and on well-drained very acid soils (Freebairn, 1980; Bolland, 1985).

Serradella is singularly efficient in utilising phosphate and potash, and where the light acid soils in the far north of New Zealand have been adequately topdressed with phosphate, potash, and lime, results are good (Halliwell, 1960).

Gladstones et. al. (1977) suggested that serradella should be sown at a shallow depth. Broadcasting or dropping the seed on to the soil surface, followed by light harrowing, is usually satisfactory.

Undersowing in a properly nodulated crop of lupins will automatically ensure good nodulation of serradella germinating the following autumn, because the strains of bacteria nodulating serradella and lupins are identical. If inoculating seed which has not been dehulled, seed should be pre-moistened with water at 0.75 litres per 12 kg of in-hull seed. The appropriate amount of peat-inoculum is mixed in an adhesive solution (50 g methyl cellulose to 1 litre of water,) for use at 1 litre per 12 kg of seed. Finally, pelleting lime is applied at the rate of 6 kg per 12 kg of seed. (Gladstones et. al., 1977).

However, Parker and Oakley (1965) thought lime pelleting of the seeds reduced nodulation of both lupins and serradella.

If satisfactory nodulation is attained in the first year, it is most unlikely that there will be

any further nodulation problems (Gladstones et. al., 1977). The rhizobia associated with serradella and lupins successfully colonize problem sandy soils, a fact consistent with the finding (Lange, 1960; Graham, 1963) that they are closely related to the organisms nodulating native (Australian) leguminous plants. Because of its apparent freedom from second-year nodulation problems and its proven ability to grow on infertile soil, serradella would appear to hold possibilities as a pasture legume for those districts where second-year-mortality is serious (Gladstones et. al., 1964). Also, Chatel et. al. (1973) and Gladstones et. al. (1977) suggested that unlike those bacteria nodulating clovers, medics, peas and vetches, the bacteria nodulating serradella and lupins can colonise and persist well in sandy soils.

#### 1.2.2.5. Problems

The pods of serradellas are usually constricted between segments, and seeds are often difficult to separate from the segments. The high percentage of hard seed is one of the most important characters in all serradellas except French serradella which has fully permeable seeds.

Unlike most other legume seeds, the seeds of serradella are enclosed in pod segments when sold and cannot be scarified in the normal manner to reduce the proportion of hard seeds and increase the germination. Commercial seed (yellow serradella) normally has an immediate germination potential of about 5 per cent, and good first-year stands can not be achieved unless very high seeding rates are used (Cariss et. al., 1967).

Dehulling of serradella seed is still used extensively only for experimental purposes, using small machines which do not have the capacity to handle commercial quantities (Bolland et. al., 1987).

### 1.2.3. Animal performance and compatibility with other legumes

Thirty years ago, serradella had become a plant of considerable economic importance in the far north of New Zealand for grazing animals and especially for dairy cows. The forage is very palatable and lambs fatten readily on this legume. Pigs and poultry also thrive on serradella. On Mr. J.C. Farnham's property, Waiharara, about 0.41 ha of serradella carried 23 milking cows for seven days during August (Halliwell, 1960). However because of difficulties with seed supply, establishment and persistence, serradella has largely been replaced by other legume species.

In Coonabarabran and Coolah Shire in Australia, it was reported that eight ewes per hectare had been run constantly on serradella pasture for 10 months Lambing rate was 120 per cent (Freebairn, 1980).

Gladstones et. al. (1977) suggested that sowing a mixture of subterranean clover and serradella in paddocks with mixed soil types had not been as successful as might be expected. When mixed with high-oestrogen subterranean clover, the serradella tended to be eaten out preferentially because of its greater palatability. If the subterranean clover was earlier maturing than the serradella (which would normally be the case), the green serradella remaining after subterranean clover maturity was subject to intense grazing just when it was at its most vulnerable stage, resulting in a failure of seed production even at light stocking rates.

When yellow serradella is sown with subterranean clover on soils where clover persists successfully, the serradella rapidly diminishes to become a minor species in the pasture. For sands of marginal depth for subterranean clover i.e. between about 0.3 and 0.5 m to the heavier subsoil, serradella appears to be useful as a pioneer species; after it has built up fertility sufficiently, subterranean clover will gradually volunteer and eventually become the dominant species (Bolland et. al., 1987).

Near Esperance, Western Australia there are sometimes shallow and deep sandy soils in the same paddock, with subterranean clover persisting on the former and serradella on the latter. But at the end of the growing season the serradella, because it is deeper rooted, tends to dry off a month or more after the clover, and because it is the only green feed left in the paddock is heavily grazed and quickly disappears. Earlier maturing serradellas may be an answer to this problem, as noted above (Bolland et. al., 1987).

Serradella is a high-protein fodder providing much needed out of season grazing and at the same time increasing soil fertility. Compared in efficiency with white clover, it will withstand low-fertility conditions (Halliwell 1960). In general, herbage production of serradella is high in poor soils, particularly deep acid sandy soils. Developing serradella is therefore most relevant to poor soil environments.

As an interesting small group of pasture legumes, the position of serradella species is changing. Their ability to quickly establish in sandy soils, improve soil fertility (efficient formation of nodules), soil erosion control, high nutritive value and palatability to livestock including pigs and poultry etc. is enough to enable them to become more and more important, even though they have some disadvantages (high percentage of hard seed, difficult to dehull, persistence, ability to compete). Serradella species have many of the attributes required to be successful pasture legumes.

## 1.3. The Problem of Hardseededness in Serradella Seed

### 1.3.1. Hardseededness of legumes

The production and the supply of high quality legume seed is confronted by several problems which must be overcome or at least minimized. One of the unresolved real problems in the production of many leguminous crops is hardseededness which is very common and found in most small seeded-legumes (Stevenson, 1937).

Impermeable seeds are common in many species of Fabaceae (legumes) as well as occurring in some species of Cannaceae, Chenopodiaceae, Convallariaceae, Convolvulaceae, Geraniaceae, Malvaceae, Solanaceae (Harrington, 1916; Rolston, 1978), Anacardiaceae (Stone and Juhren, 1951; Rolston, 1978), and Rhamnaceae (Gratkowski, 1962; Rolston, 1978). Of 260 legume species examined, 85% had some or all impermeable seeds (Guppy, 1912; Rolston, 1978).

Seeds that do not absorb water and germinate, when placed in wet or moist surroundings, are called hard seeds. Hardseededness is caused by the impermeability of the seedcoat to water. When the seedcoat is made permeable to water by some means, hard seeds show the same water absorption and germinability as normal seeds (Koller, 1964; Barto and Crocker, 1984; Li, 1989).

Impermeable seed coats permit extension of life to many seeds so that they are distributed in time as well as in space (Crocker and Barton, 1953; Rolston, 1978). Not only do impermeable seeds remain viable for a long time, but under natural conditions increments of a seed population become permeable to water and germinate in successive intervals (Williams and Elliott, 1960, Rolston, 1978).

#### 1.3.1.1. Structure of the seed coat

The seed coat usually consists of three layers: an outer layer of Malpighian cells (epidermis), a middle layer of osteoscleirid cells, and an inner layer of nutrient cells. All these three layers originate from the outer integument while the inner integument is usually absorbed during seed development. A wax-like cuticle layer caps the malpighian cells and a residue layer of endosperm cells (sometimes plus the residue of the inner integument) is under the nutrient cells. The malpighian cells are long and relatively narrow with blunt or rounded ends. The lumina in these cells are convoluted, being relatively large at the base and small or occluded near the tip of the cells. Malpighian cells are arranged perpendicularly to the

surface of the seed coat and just below the cuticle layer. They have four characteristics: (a) a mucilage stratum, often with a cap or cone; (b) an outer rod-like structure with narrow lumina; (c) an inner columnar stratum with wide, nucleate cell-lumen; (d) a light line separating b and c. The malpighian cells are lignified and may contain tannin and pigments. The cuticle layer, of variable thickness, follows the contours of the Malpighian cells and penetrates between the caps of these cells. The middle layer of the cells in the seed coat varies in thickness. The cells in this layer are mostly thick-walled and tend to be loosely jointed with air spaces between cells. The third layer of the seed coat is generally less well defined than the outer two layers. The cells in this layer are usually collapsed nutrient cells (called parenchyma cells) (Corner, 1951).

In view of the construction of the three layers of the seed coat and from studies on it, it is assumed, in many cases, that the Malpighian cell layer (epidermis) is the main barrier to the penetration of water (Homly, 1932; Martin and Watt, 1944; Watson, 1948; Pandey, 1955; Ballard, 1973).

When the seed breaks from the funicle, an abscission layer is developed so that the hilum is clearly defined (Corner, 1951). In the long axis of the hilum, there is a groove in the funicular tissue coinciding with a fissure known as the hilar valve in the epidermis of the seed coat. The fissure is the passage for water and gas in the ripe seed (Hyde, 1954).

The hilum acts as a hygroscopically activated valve, opening when the atmospheric relative humidity is low to allow moisture loss from the seed, and closing when the relative humidity is high (Hyde, 1954).

The strophiole is a narrow elongated depression, surrounded by a raised border close to the hilum on the side opposite the micropyle (Hamly, 1932; Martin and Watt, 1944). It appears to not have much to do with the development of hardseed, but it has an important role in maintaining the hardseededness. The strophiole is often the initial place where water

enters the seeds (Hamly, 1932; Quinlivan, 1968).

The function of the micropyle is not very clear in hardseededness.

The testa and its structure, the hilum, micropyle, strophiole and chalaza have all been implicated as barriers to water or as area of weakness where imbibition occurs (Rolston, 1978).

### 1.3.1.2. Development of hardseed

Based on the studies of hardseed development in lupin, white clover and lucerne by Lebedelf (1943), Hyde (1954), Gladstones (1958) and Quinlivan (1968), the development of hardseed appears to be a continuous process. The development of impermeability of the seedcoat is governed by the moisture content of the seed. As the seeds ripen on the parent plants, water lost from the seed passes through the epidermis and its covering cuticle. All the seed coats appear totally permeable before the seed moisture content falls below 25%. Below 25%, the epidermis begins to resist the movement of water and the impermeability develops until the seed coat becomes entirely impermeable at approximately 14% moisture content.

At this stage, the loss of water is mainly via the seed coat, although the hilum has began to function at this stage. After the seed coat becomes totally impermeable, the further loss of water is controlled by the hilum (Hyde, 1954).

Lebedeff (1947) exposed beans to 10 moisture levels, giving seed moisture contents ranging from 14.11% to 5.39%. The 14.11% moisture content beans had 1% impermeable seeds compared to 90% impermeable seeds for the 5.39% moisture content. Each reduction in the moisture content increased the percentage of impermeable seeds, and the seeds remained hard for a longer period of time. The moisture content of the seed was determined by the relative humidity (RH) and the time of exposure and the hard seed content increased

as seed moisture decreased (Harrington, 1949; Rolston, 1978). In soybeans, sites with low relative humidity and high temperatures developed more hard seed (Rolston, 1978).

Barrett-Lennard and Gladstones (1964) found that hardseededness (impermeability) of Western Australian serradella (Ornithopus compressus L.) was found to be governed by seed moisture content. At storage humidities of 76% R.H. and more, all initially soft seeds remained soft, while at 44% R.H. or less and in the open laboratory, all seeds became fully hard. Storage at 52% and 66% R.H. resulted in semi-hardness and delayed germination.

The differences in texture and structure between permeable and impermeable seed coat have been investigated in several plants. There seems to be little difference in physical and chemical structure between these two types of seed coats (Stevenson, 1937).

Raleigh (1930) and Hyde (1954) suggested that the shrinkage of the seed coat during ripening (or during processing and storage) was the main cause of the impermeability. During seed ripening the seed shrinks due to loss of water, the malpighian cells became pressed together, the lumina of the cells are obliterated by the shrinking force, and the seed coat becomes an impermeable layer. The further drying and shrinkage of the seed made by the hilum increases the compression of the malpighian layer, and intensifies the impermeability.

### 1.3.1.3. Factors causing hardseededness

It is agreed that at least several factors are involved in the development of hardseededness, but seed moisture content is considered as the main factor controlling the impermeability of the seed coat. In addition, genetic factors are regarded as another main factor as well. Other factors, such as relative humidity and temperature are related to the moisture content. Fertilizer application and sowing time etc. can also affect the development of hardseed. In lucerne, the hybrid Medicago media had 20% impermeable seed compared to the parent types, M. sativa and M. falcata, which had 5% and 56% impermeable seeds, respectively (Mijatocic,

1971; Rolston, 1978). Lebedeff (1947) concluded that only a few genes are involved in the differentiation of hard- and soft-seeded selections of Phaseolus vulgaris. Impermeable seed in blue lupine (Lupinus angustifolius) is controlled by a single dominant gene, whereas soft-seededness in two cultivated varieties was controlled by an allelic recessive pair (Forbes and Wells, 1968; Rolston, 1978). In Vicia, Donnelly et. al. (1972) reported two gene inheritance, where gene A was a simple dominant for hard seed and gene B was dominant for soft seed when the A locus was a homozygous recessive aa.

In serradella, O. sativus maintains completely soft seed during seed development and maturity in the same environment in which other species such as O. compressus develop hard seeds. This is a genetic difference.

Soil fertility may play a role in the development of impermeable seeds. Several experiments have shown small changes in the impermeable seed content of species treated with fertilizer. James (1949a) and James and Bancroft (1951) increased the hard seed content of crimson clover with CaCO3, while potash (110 kg/ha, K) reduced impermeable seed content. El Bagoury and Niyazi (1973) recorded a depression in impermeable seeds of Trifolium alexandrinum with a combination of potassium sulphate (476 kg/ha) and ammonium sulphate (476 kg/ha) but found no effect from the potassium sulphate alone. In an earlier experiment, El Bagoury (1957) increased the impermeable seed content of Vicia faba from 10% to 20% with potassium sulphate (238 kg/ha). Soil fertility appears to play a minor role in the development of impermeable seeds (Rolston, 1978).

The development of hardseededness is complex and may be caused by multiple factors, both interspecific (genetic) and intraspecific (genetic and environmental). For some species, the presence or degree of hardseededness depends upon their genetic constitution, but environmental factors are dominant for other species. At present, it is not always easy to determine the relative contribution by either.

### 1.3.1.4. Methods of breaking hard seed

Various treatments that will induce hard seeds to germinate have been known for many years, and the early methods have been reviewed by Porter (1949). Table 1-4 lists examples of a variety of treatments on the germination of impermeable seeds (Rolston, 1978).

Softening of hard seeds can occur in two ways (Rolston, 1978). One is through artificial softening such as chemicals, enzymes, pressure, percussion, scarification, freezing, heating and radiation (Table 1-4). The other is by natural softening. In nature, the seed coat may be broken down or punctured by mechanical abrasion, especially during cultivation, passage through the digestive tract of animals and birds, by fire, and by high temperatures and temperature fluctuations which are a major factor in softening seeds of winter annuals in Australia (Quinlivan, 1970a) and California (Williams and Elliott, 1960). In subterranean clover, Ornithopus compressus, Lupinus varius, and Stylosanthes humilies, natural softening under temperature fluctuations does not appear to commence until the moisture content of the seeds is low (Barrett-Lennard and Gladstones, 1964; Quinlivan, 1971a), e.g., less than 8.5% in Lupinus varius (Quinlivan, 1968; Rolston, 1978).

Natural reversibility under high RH also occurs. Subterranean clover seeds with 10% moisture (dry weight basis) are conditionally hard and soften slowly in a humid atmosphere while seeds with less than 8.5% moisture are absolutely hard and will not soften in moist conditions, although temperature fluctuations are effective in softening. Thus, germination of subterranean clover in the field will depend on the degree of desiccation of the seed and the maximum temperature (Quinlivan, 1971a; Rolston, 1978).

### 1.3.2. Effects of hardseededness in serradella seed

Hardseededness affects seed quality greatly by lowering germination which influences the sowing value and ability to rapidly establish. Many efforts have been made to depress

## Table 1-4

Examples of the Effects of Different Treatments on Various Impermeable Seeds

| Treatment | Species | % Permeable | | Author | Date |
|---|---|---|---|---|---|
| | | Untreated | Treated | | |
| ACID | | | | | |
| $H_2SO_4$(conc), 15 min. | *Centrosema pubescens* | 28 | 88 | Win Pe *et al.* | 1975 |
| $H_2SO_4$(conc), 30 min. | *Cercis canadensis* | 10 | 96 | Afanasiev | 1944 |
| $H_2SO_4$(conc), 30 min. | *Colutea istria* | 5 | 98 | Koller & Negbi | 1955 |
| $H_2SO_4$(conc), 30 min. | *Convolvulus lanatus* | 0 | 84 | Koller & Cohen | 1959 |
| $H_2SO_4$(18N), 30 min. | *Coronilla varia* | 29 | 84 | Brant *et al.* | 1971 |
| $H_2SO_4$(conc), 3 hr. | *Rhus ovata* | 3 | 62 | Stone & Juhren | 1951 |
| $H_2SO_4$(conc), 30 min. | *Strophostyles helvola* | 29 | 79 | Hutton & Porter | 1937 |
| SOLVENTS | | | | | |
| Absolute ethyl alcohol, 82 hr. | *Acacia constricta* | 9 | 88 | Barton | 1947 |
| Absolute ethyl alcohol, 20 hr. | *Convulvulus arvensis* | 15 | 90 | Callihan | 1961 |
| Absolute ethyl alcohol, 72 hr. | *Gymnocladus dioica* | 4 | 92 | Barton | 1947 |
| PRESSURE | | | | | |
| 2000 atm, 10 min. | *Melilotus alba* | 24 | 83 | Davis | 1928 |
| 2000 atm, 1 min. | *Medicago sativa* | 48 | 83 | Davis | 1928 |
| PERCUSSION | | | | | |
| Shaken, 10 min. | *Amorpha fruticosa* | 46 | 73 | Hutton & Porter | 1937 |
| Shaken, 20 min. | *Cladrastis lutea* | 6 | 82 | Barton | 1947 |
| Shaken, 6 hr. | *Colutea istria* | 5 | 90 | Koller & Negbi | 1955 |
| Shaken, 4 hr. | *Convolvulus lanatus* | 0 | 94 | Koller & Cohen | 1959 |
| Shaken, 10 min. | *Lespedeza capitata* | 4 | 84 | Hutton & Porter | 1937 |
| Shaken, 5 min., 550 oscill/min. | *Trifolium subterraneum* | 12 | 63 | Ballard & Grant Lipp | 1965 |
| SCARIFICATION | | | | | |
| Mechanical | *Astragalus cicer* | 42 | 93 | Townsend & McGinnies | 1972 |
| Mechanical | *Centrosema pubescens* | 28 | 85 | Win Pe *et al.* | 1975 |

(Continued)

Table 1-4 (*Continued*)

| Treatment | Species | % Permeable | | Author | Date |
|---|---|---|---|---|---|
| | | Untreated | Treated | | |
| Filed | *Convolvulus arvensis* | 6 | 98 | Callihan | 1961 |
| Grindstone | *Crotalaria occaleuca* | 10 | 58 | Jones | 1971 |
| Mechanical | *Desmodium uncinatum* | 2 | 56 | Keya & van Eijnatten | 1975 |
| Hole drilled through seed coat | *Rhus ovata* | 3 | 74 | Stone & Juhren | 1951 |
| Abraded | *Trifolium hybridum* | 4 | 81 | Nakamura | 1962 |
| Abraded | *Trifolium repens* | 11 | 51 | Nakamura | 1962 |
| FREEZING | | | | | |
| -195.8°C | *Coronilla varia* | 16 | 68 | Brant *et al.* | 1971 |
| -15°C, 1 hr. | *Medicago sativa* | 5 | 21 | Midgley | 1926 |
| HEATING | | | | | |
| Water, 70°C, 30 sec. | *Abutilon theophrasti* | 20 | 98 | LaCroix & Staniforth | 1964 |
| 95°C, 6 min. | *Abutilon theophrasti* | 34 | 85 | LaCroix & Staniforth | 1964 |
| Water, 90°C | *Ceanothus sanguineus* | 5 | 90 | Gratkowski | 1973 |
| Boiling water, 30 sec. | *Coronilla varia* | 17 | 49 | Brant *et al.* | 1971 |
| 510°C, contact heat | *Medicago sativa* | 34 | 78 | Lunden & Kinch | 1957 |
| 60°C, 20 min. | *Medicago sativa* | 37 | 68 | Stetson & Nelson | 1972 |
| 100°C, 5 min. | *Rhus ovata* | 3 | 34 | Stone & Juhren | 1951 |
| RADIATION | | | | | |
| Glow discharge, 3 min. | *Gossypium hirsutum* | 38 | 73 | Goodenough *et al.* | 1970 |
| Glow discharge, 60 $H_z$, 3 min. | *Gossypium hirsutum* | 3 | 58 | Stone *et al.* | 1973 |
| IR, 1.1 sec. | *Medicago sativa* | 48 | 83 | Nelson *et al.* | 1968 |
| RF, 39 $MH_z$, 5.8 sec. | *Medicago sativa* | 48 | 88 | Nelson *et al.* | 1968 |
| Microwave 2450 $MH_z$, 80 sec. | *Medicago sativa* | 32 | 75 | Stetson & Nelson | 1972 |
| SEED STORAGE | | | | | |
| Drying, over $CaCl_2$ | *Abutilon theophrasti* | 12 | 72 | LaCroix & Staniforth | 1964 |
| Moist cabinets, 20 months | *Medicago truncatula* | 2 | 48 | Kirchner & Andrew | 1971 |

hardseededness and improve germination rate and uniformity.

Most serradella (Ornithopus) spp.,have a high percentage of hardseeds e.g O. compressus with > 90%. The exception is O. sativus which has completely soft seeds. The pods of serradella are small, cylindrical and curved upward. These curved pods when dry break into segments containing the yellow seeds, which are difficult to separate from the segments (Halliwell, 1960).

### 1.3.2.1. Seed processing and imposed dormancy

The pods of cultivars Pitman and Uniserra (Ornithopus compressus) are segmented, each segment usually containing a seed. At the end of the growing season the mature dry pods readily break between segments and eventually drop onto the soil surface. Farmers generally harvest the pods with a conventional crop harvester soon after the plants have matured but before most pods have broken up and dropped (Bolland et. al., 1987).

Commercial serradella seed is purchased as undehulled pod segments; 90% or more is hard-seeded (impermeable), because the podded seed is not scarified whilst being harvested (Bolland et. al., 1987).

The poor germination of serradella, owing to its high degree of hard-seededness, has been one of the main factors limiting its expanded commercial use in Western Australia. However work by Barrett-Lennard and Gladstones (1964) showed that considerably higher germination should be obtainable in practice. It was found that germination was prevented by (a) simple impermeability of the seed coats and (b) a dormancy-inducing substance present in the pods, particularly in immature pods (Gladstones et. al., 1964).

Barrett-Lennard and Gladstones (1964) suggested that the seed dormancy imposed by the husk in Western Australian serradella is caused by a water-soluble substance which is leached

from the husk and passes into the seed during imbibition. Such mechanisms, whereby germination is inhibited by water-soluble substances present in parts of the dispersal unit other than the seed itself, are not uncommon. Koller (1964) considered that this occurrence is an adaptation to arid or semi-arid climates where rainfall is the most variable environmental factor. The requirement that an inhibitory substance be leached out would give the seeds some protection against germination should they become imbibed following ineffective rainfall.

Because of the presence of dormancy-inducing substances in the pod walls, together with the hardness of the seed coats, which are protected by the pod from scarification during threshing, the germination of commercial serradella seed has often been as low as 2 to 10 per cent (Weeldenburg et. al., 1969).

Laboratory tests have shown that germination can be increased to nearly 100 percent, by removing the seeds from the pod segments (dehulling), followed by scarification (Weeldenburg et. al., 1969). A commercial dehulling machine was developed to produce commercial Pitman and Uniserra seed. However, to most it seemed cheaper to buy the podded seed and there was little demand for the dehulled seed (Bolland et. al., 1987).

### 1.3.2.2. Methods for breaking hardseed of serradella and establishment in the field

Barrett-Lennard and Gladstones (1964) proposed that substantially improved germination of Western Australian serradella seed could be achieved on a commercial scale in at least two ways. The first would be complete hulling and scarification of the seeds. This would overcome both seed impermeability and husk-imposed physiological dormancy. Although hulling and scarifying on a commercial scale could present difficulties and lead to some seed losses, their experience in the laboratory suggested that the difficulties might not be insuperable. The second method would be to expose the pod segments to alternating high and low temperatures. This could be done either in temperature-controlled ovens, or simply by exposing the seed to the sun for several months during summer. For this method to be

properly effective the seed would have to be harvested fully mature, in order to avoid the apparently persistent dormancy-inducing influence of the immature pods.

Other methods have been used, such as storing the pod segments in a thin layer (2-3 cm) under a black plastic sheet during summer; boiling the pod segments for 2-3 min; spreading the pod segments on the surface of soil under a crop in early summer, when most of the hard seeds will soften during the following summer and germinate at the beginning of the next growing season. This is an effective and convenient method for establishing yellow serradella. For example, Bolland (1986) reported that Pitman serradella could be successfully established on deep sandy soil by broadcasting pod segments or dehulled and scarified seed. Because of hardseededness, most podded seed (95%) did not germinate under a wheat crop but most of these hard seeds softened during the following summer and germinated to emerge (60-80%) in the first pasture after the crop, albeit at relatively low densities. By contrast, most of the dehulled seed had germinated and produced plants under the crop. However, these plants must have produced only a few seeds under the crop because the serradella did not persist in subsequent years. The wheat plants which produced 4-6 t/ha of dry matter would have markedly reduced the amount of light reaching the serradella growing underneath, particularly in spring, which probably reduced serradella growth and seed production. Moreover, both the wheat and serradella plants would have competed for depleted soil water reserves in spring when both were flowering and producing seed.

Also, dehulled and scarified seeds could be sown in the crop stubble just before or after the break of the season in the pasture year. Presumably such seed would be considerably more expensive than pod segments, but this added cost would at least be partly offset by a considerably reduced risk of seedling loss from false breaks which could beset the pod segment techniques (Bolland, 1986).

At present, the methods for dehulling and scarifying serradella seeds and field establishment are ineffective, and need to be further improved because of the cost of

commercial scale processing, and damage during seed processing. This could have a considerable impact on both the cost and the ease of field establishment, especially if it is possible to improve the processing technique (machines) and reduce cost.

## 1.4. Evaluation of a New Herbage Legume

### 1.4.1. General

In the light of 20 years' experience, evaluation of genetic resources in germplasm collections is due for reappraisal. It has been a widely accepted axiom in earlier genetic resources literature that evaluation is an essential preliminary to utilisation (Frankel & Bennett, 1970).

Evaluation was seen as an organised and institutionalised activity resulting in information which comes to the user (the breeder) as standardised and computerised documentation (Finlay & Konzak, 1970). Also, Hyland (1970) pointed out evaluation aims to reveal potentially useful variability. Evaluation was the responsibility of the curators of germplasm collections (Frankel, 1989).

The description of accessions and the recording of the information in databases are aspects of genetic resources work which have progressed slowly. The large number of samples now conserved in genebanks makes their comprehensive description and evaluation a formidable task (Williams, 1989).

The controlled use of 'wild' plant germplasm is largely dependent upon accurate description and taxonomic identification, followed by comprehensive evaluations by competent authorities. The thoroughness of such evaluation depends upon the number of accessions under observation at a given time, the diversity of genera or species, and the availability of specialists. Transferring a plant from its original habitat to a different location

and environment often results in phenotypic differences and in many cases unconscious selection results in genetic change. Thus descriptive records, from the time of original discovery and throughout the evaluation period become highly important. They should be prepared for long-term reference, and assembled methodically for computer adaptation (Hyland, 1970).

With most wild or primitive plant collections, this necessitates an initial evaluation, generally in nurseries, followed by a more detailed field study to characterize growth habit and general potential. For initial tests a favourable environment comparable to the original habitat of the species is usually provided. However, abnormal conditions may reveal useful characteristics. Therefore plantings should not always receive optimal environmental treatment. Locations should be chosen where competent pecialists (agronomists, geneticists, pathologists, etc.) can collect specialized information (Hyland, 1970).

In recent years there has been an upsurge in interest in evaluating the potential of numerous alternative plant species. Much of the research has concentrated on the soil conservation potential of alternative species in revegetation of tussock grasslands (Wills, 1983; Rys et. al., 1988). However, many of the species evaluated may have potential under more intensive pastoral systems (Rys et. al., 1988).

Forage evaluation occurs in a series of stages. It begins from the time that people recognise or introduce a new plant species, progresses to the accumulation of knowledge of performance capabilities, then ends when the new plant species is reasonably and appropriately used and improved in terms of its particular purposes.

Characteristics that will influence the adoption of alternative species include factors such as growth habit, adaption to grazing, speed of establishment, alternative end uses and forage characteristics (Wills, 1983). However, the widespread use of alternative forage species in pastoral agriculture ultimately depends upon achieving a high, sustainable yield and annual

acceptability and performance (Rys et. al., 1988).

In principle the procedures adopted in evaluating plants for forage are universal. Briefly, they are: to determine what improvement is needed in an animal production system; to gain confidence that the plant is superior to existing forages in quantity or quality and will propagate satisfactorily; and to be assured that it will, when used appropriately, improve the total productivity of the farming system (Wheeler, 1981).

### 1.4.2. Forage evaluation schedules and criteria

Mochrie et. al. (1981) suggested a protocol for evaluation of new forages outlined below:

A theoretical model for development and evaluation of a new forage

| Plant | Phase | Bioassay-animal |
|---|---|---|
| | I | |
| Collection of material and conception of its potential agronomic characteristics, and quality (chemical) | | IVDMD* for quality here and in subsequent phases |
| | II | |
| Persistence - geographic (climatic and edaphic), insect and disease resistance, yield and growth response to simulated grazing (limited clipping) | | Animal preference - to assist cultivar selection, quality (intake, oesophageal, etc.) estimates, limited plant response to grazing (regrowth and persistence) |
| | III | |
| Detailed management - stand establishment and maintenance, yield, growth rate and seasonal distribution in response to fertility and clipping routines | | Qualitative animal response - daily gains, quality of consumed forage, plant regrowth and persistence to continuous or intermittent defoliation by animal, preliminary estimate of economic potential |

| | | |
|---|---|---|
| | IV | |
| Seed increase or expanded vegetative source | | Quantitative animal evaluation-production/ha under management variables (fertility, grazing method, etc.) and resulting forage quality and response to various defoliation schemes, more extensive economic evaluation |
| | V | |
| Release and on-farm implementation - education of user on establishment and maintenance of stands, observe for possible complications with long term exposure (pests, disease, unusual climate, etc.) | | Observation of response to herd or flock utilization - including the development of roles in grazing or feeding systems |
| | VI | |
| Refinement and continued improvement - evaluate special management practices devise ways to alter distribution of growth, and develop new related genetic material to overcome deficiencies | | Refinement - animal response to plant management practices |

* IVDMD = in vitro dry matter disappearance

Before evaluating a new pasture plant, three things should be considered (Wheeler, 1981):

1. The physical environment (rainfall characteristics, temperature range, soil information and erosion hazards where the forages are being evaluated)
2. The farming system used currently and envisaged for the future.
3. The candidate species and genera. Included in this category would be informationgained from literature or from colleagues, on the range of pH likely to be tolerated by the

species, its nutrient requirements, the rainfall it needs, its value for animal production and the probability of it containing substances injurious to animals.

Many different criteria have been used, normally in combination, to evaluate forages for animal production. The basic evaluation criteria (Wheeler, 1981) are:

1. Ease of establishment. A plant is unlikely to gain acceptance by farmers if it is difficult to establish from seed or vegetatively.

2. Yield and seasonal distribution of dry matter. Visual ranking of measurements of fresh yield may be acceptable in the initial stages. If necessary exact moisture contents may be quickly and cheaply determined from the loss in weight after heating a sample in vegetable oil to c. 155°C. Plants should be grown both with and without fertilizer. Note should be taken of the susceptibility to disease or insect attack, frost and drought in the course of dry matter production trials.

3. Digestibility. The simplest system for estimating DM digestibility would appear to be the use of nylon pouches suspended in the rumen of a beast maintained on a uniform diet of comparable forage.

4. N content. An assessment of the N content of potential forages is highly desirable. There appear to be no satisfactory means of estimating N content apart from the standard laboratory procedures.

5. Satisfactory nodulation of legumes. Many tropical legumes are promiscuous in their rhizobial associations and may not need inoculation but candidate legumes should be checked for effective nodulation in the soils for which they are intended.

6. Animal response. It is generally necessary to determine the production response by

animals to the proposed plants. Measurement of animal production can reveal mineral and nutrient deficiencies in the forage. Moreover, demonstrable differences in animal production are particularly effective in encouraging farmers to adopt new forage production practices.

In the initial stage, the criteria are purely agronomic and adapted to the appraisal of relatively large numbers of candidate plants. Intermediate stages involve increasing use of the animal and the final stage relies heavily on animal production criteria (Wheeler, 1981).

### 1.4.3. Evaluation techniques and data collection

Usually, an initial evaluation of forage legumes is based on the study of agronomic properties such as growth habit, yield (dry matter), nutrient components (crude protein, fat, carbohydrate and trace elements), resistance to pests and diseases, plant adaptation to climate and edaphic (soil) factors, capacity of forming effective nodules.

#### 1.4.3.1. Growth habit

Growth habit of the plant will determine its ultimate utilization (for hay, grazing, soil erosion control etc.). Correct description and identification of important characters is essential to properly reflect the physical properties of the plant evaluated. The data collected include morphological and biological characteristics, such as life form (annual, biennial, perennial), features of plant surfaces (pubescent or glabrous), the number of leaves/node, the number and shape of leaflets, height of plant, inflorescence type, flowering time, colour and size of flower, colour, size and shape of pods and seeds, whether pods are dehiscent or not after maturation etc. All description work is based on observation and specimen analysis.

### 1.4.3.2. Adaptability and resistance

A legume will only be accepted by the user if it has good adaptation to the climate and soil environment, and a good resistance to pests and diseases. Also, the ability of the plant to withstand grazing can be important in many farming systems. The growth and survival characteristics of a legume determines its potential for development. This potential reflects in persistence, growth rate, capability of reproduction and resistance to pests and diseases. All of this information is based on field observations, or special trials carried out on the plant.

### 1.4.3.3. Yield and seasonal distribution of dry matter

Yield is often one of the most important characters to be evaluated in forage legumes. The dry matter distribution of the legume in different seasons determines its form of utilization. The measurements of yield can be divided into two types, total and seasonal yield, and the measurements depend on the experimental requirements. Usually, the samples are collected directly from the experimental plots. For the determination of dry matter, two methods may be used, oven-dried (usually at 80°C), or freeze-dried (Burns, 1981).

Moisture in herbage will vary with variety, species, season and stage of growth and degree of wilting. Differences in cellular structure of leaves and stems widely affect drying rates (Burns, 1981).

### 1.4.3.4. Nutrient composition

For the determination of 21 elements including nitrogen a direct reading from an emission spectroscopic method (Quantometer) can be used. This employs a briquetting technique using finely ground forage and results can be obtained within 24 hours of collecting the forage sample (Minson, 1981). The procedure is that the plant material is dried at 80°C for 12 hours and ground to a very fine powder in a Spex shattermill for five minutes. The plant material

is then mixed with powdered carbon, lithium carbonate and cellulose and pressed into a briquette at 70,000 p.s.i. The briquette is excited with a high voltage condensed arc in an atmosphere of argon and the spectral lines ranging from the vacuum ultra-violet to the near infra red recorded and automatically converted into concentrations of the 21 elements (Minson, 1981).

### 1.4.3.5. Nodulation

The ability of suitable rhizobia to colonize a new site has a profound influence on the colonizing ability of a legume; similarly, the ability of the legume to adapt genetically to suit the Rhizobium strain may be significant (Mathison, 1983).

The mere presence of nodules is no guarantee of benefit to the plant. Effective symbioses are indicated by large pinkish nodules attached to the upper laterals and the top root, and a dark green foliage. Ineffective symbioses are indicated by the presence of very numerous small greenish white nodules scattered distally along secondary and tertiary roots, with nitrogen-deficiency symptoms often reflected in unthrifty yellowish foliage (Allen et. al., 1981). Usually, the success of nodulation can be observed directly by carefully digging up plants.

### 1.4.3.6. Palatability

The palatability of a legume to animals is significant for future utilization. It depends upon the characteristics of the surface of the plant (pubescent or glabrous), the texture (woody or not), and chemical components contained by the plant itself (toxicant, peculiar smell). All these can affect the feeding value of the legume.

The comparison of palatability, in general, is direct i.e. grazing the animals in the paddock with several different species of legumes, observing the patterns of animal grazing and

consumption of each legume. Finally, the degree of grazing is evaluated.

### 1.4.4. Information communication and application

After collection of wild plant species, it is essential to make an initial evaluation. The purposes of the initial evaluation are to provide some information and ideas for use by breeders and farmers in order that the species being evaluated may be introduced into a new region for further evaluation. Breeders may select some types as material for breeding new cultivars.

It has been recommended that breeders, advisers and merchants should be far more aware of farmers' needs when communicating on herbage cultivars. How can this be achieved? Communication should be considered a two way exchange of information and ideas. In the long term, to improve the rate of herbage cultivar adoption there must be better communication between farmers and plant breeders before a breeding programme starts (French & Simmonds, 1985).

Forage evaluation is a comprehensive concept covering many aspects. Any sort of evaluation has different requirements for different purposes. A complete evaluation is a gradual procedure and can be divided into several stages. A successful legume should contribute to increased soil nutrients and animal production.

The evaluation of primitive plant material requires competent personnel and sufficient time to do the task thoroughly. It becomes more difficult when large numbers of accessions are to be screened, particularly if the accessions involve several genera or species. One of the most controversial and difficult tasks in the evaluation phase is the maintenance of the genetic composition of cross-pollinated plants during testing (Hyland, 1970).

### 1.4.5. Multiplication

Frankel (1970) pointed out that the need for multiplication may arise any time after a collection is made. It is nearly always attended by trouble, risk or loss--trouble in growing and harvesting, risk of contamination, of infection, or loss through natural selection--the nature and extent of these difficulties depending on a number of factors:

(i) size of original sample. The larger the original sample, the easier and safer it is to test, conserve and multiply. Collections of wild material are often so small that they must be multiplied even for conservation, let alone evaluation.

(ii) reproductive system. Prevention of unwanted hybridization during multiplication is as essential as it is difficult.

(iii) environmental conditions should favour survival and reproduction of all components. This necessitates careful site selection, and sometimes duplication of sites to reduce the bias of natural selection at any one site. Some wild plants are exceedingly hard to grow outside their natural range.

(iv) protection against fungal, bacterial or virus diseases for all multiplications is necessary.

A number of procedural principles apply generally:

(1) Splitting versus pooling. A decision has to be made in each case whether, and to what extent, a collected sample should be separated into component parts if there are recognizable variants, whether it should be treated as a unit, or whether it should be pooled with other samples related to it ecologically or genetically. This decision should not be made solely or mainly on grounds of convenience, for it cannot be

undone at a later stage unless reserves have been stored.

(2) Conservation versus evaluation and distribution. It is advisable to make

provision for conservation at the earliest stage, i.e. from the original material wherever possible. If multiplication of material for conservation is required, every precaution should be taken to preserve its integrity as far as this is possible. Larger quantities are needed when multiplication is made for evaluation and for distribution to other institutions.

(3) Methods and facilities. Methods and facilities for multiplication of introductions are not different from those commonly used in the breeding of the respective plants. Similarly, facilities required correspond to those of well-equipped plant breeding stations. These may include greenhouses, isolation houses, and ample facilities for the control of fertilization. There must be an adequate and experienced professional and technical staff (Frankel, 1970).

# CHAPTER 2

# MATERIALS AND METHODS

## 2.1. Experiment 1. Identification and evaluation

This experiment was divided into two parts and carried out (i) in a glasshouse at DSIR Grasslands, Palmerston North and (ii) in a fenced field at Aokautere, a suburb of Palmerston North. The total number of accessions tested was one hundred and seven, 51 of which had been collected from South-West Europe (wild material) by DSIR Grasslands staff (Dr. Margot B. Forde and Dr. Sidney Easton) in 1986. The other 56 were varieties and accessions introduced from other countries and/or collected in New Zealand (Table 2-1).

Samples of seed segments (most around 100 segments, but some not more than 50 because of the limited original number of segments collected) of the 107 accessions were germinated in early April, 1990. Most of them had not been dehulled or scarified. To germinate they were placed on moistened filter paper in petri dishes and kept in a germinator at a constant temperature of 20°C. After 14 days, most segments and seeds had germinated and possessed radicals around 1 cm long. These germinated seeds and segments were then transferred into trays (42 x 30 $cm^2$) containing potting media of General Mix - D2 (a potting medium consisting (per cubic metre) of 60% Peat, 40% Sand, Osmocote 3-4 month 600 g, Osmocote 8-9 month 2400 g, Dolomite Lime 3000 g, Superphosphate 1000 g, Fritted Trace Elements 125 g). One tray was set up for each accession (46 seedlings/tray).

The trays were kept in a glasshouse (15-22°C) until the seedlings grew to 10 cm high. Then, they were removed from the glasshouse to harden off during a harsher environment in winter (June and July). In early spring, from 31st July to 3rd August, thirty seedlings of each

accession were transplanted into the field at Aokautere. Three replicates of 10 seedlings were set out. Plot size for each replicate was 5 x 2 $m^2$ and the distance between seedlings was 100 cm. Accessions were arranged randomly within blocks except for the first block (when they were arranged in series). The total area for the experiment was 3825 $m^2$. The soil type was a Manawatu Silt Loam with a pH of 6.0 and soil test results of Olsen P 40, Ca 1482, Mg 158, Na 38.1 and K 637.5 ug/g respectively. There was no irrigation. Plot management consisted simply of weeding by means of a rotary hoe when seedlings were small and then hand weeding three times during the growing season.

A subset of 5 seedlings of each accession was left in the original trays and used for seed multiplication, observation and a record of morphological characteristics. These seedlings were kept in an unheated glasshouse to ensure the safe preservation of the initial accessions, in case seed multiplication outside was not successful. These plants were watered every day. Observation of morphological characteristics was carried out on the plants in the glasshouse every three days. For each characteristic a measurement was made on three different plants (3 samples/plant) in each accession. Plants were measured for height and absolute stem length when the first flower appeared on them. Healthy basal adult leaves were measured for length and classified for shape and number of leaflets during the flowering period. Inflorescences, flowers, peduncles and bracts were described and recorded during the flowering period. Legumes and seeds were described and recorded at maturity.

Some characteristics of agronomic importance such as branch density, inflorescences/branch and flower number/inflorescence were also recorded in the glasshouse. Other agronomic characteristics such as growth habit, flowering time and days to maturity were recorded in both the glasshouse and the field. Field observations were carried out on each replicate except for flowering time when data were recorded for each plant. 1-3 scales were used to describe relative growth habit ( 1 = prostrate 2 = semi-prostrate 3 = erect ) and stem density ( 1 = very dense >50 stems/plant; 2 = dense 10-50 stems/plant, 3 = a few stems < 10 stems/plant ) for the four species. Flowering time was the date at which the first fully

open flower was seen. To determine inflorescences/branch and flower number/inflorescence, samples of 30 inflorescences were taken at random from the full set of accessions of each species. Five plants were dug randomly from the accessions of each species in the field in order to observe root nodulation and similar observations were also performed on the plants in the glasshouse by carefully removing the soil surrounding the roots. Days to maturity was determined by calculation of the number of days from first flower appearance to harvest of the pods.

During late November and early December in 1990, the seeds in the glasshouse ripened and were harvested. Determination of pod maturity was by colour. They were ready to harvest when the pod colour changed from green to yellow or yellow brown. All five plants per tray were taken out by hand and placed in a paper bag, then dried in a ventilated unheated glasshouse until ready for threshing. Seed cleaning involved threshing pods with a belt thresher (DSIR Grasslands), and using a screen (6.3 mm) to sift large pieces out of the sample. The mixture of pods, small stems and leaves was then passed through a superior fractionating aspirator (Carter-Day, USA) to separate light material from pods. Following that procedure, light, immature pods and heavy material such as particles of stone and soil were separated over a gravity table (Westrup, Denmark). The sample was then once more sifted with a screen (4.5 mm or 4.8 mm) to take out any immature pods. Finally, the samples were blown with an air flow cleaner (Duty Master, Elec. and Eng. Co. USA) and cleaned segments were stored in a small plastic bag.

The pods of serradella from the field were harvested by the same way i.e when the pod colour became yellow and some of them had dropped on to the ground. Harvested material was placed in one sack for each plot (10 plants/sack), and was stored in a shady and ventilated shelter for drying. Seed cleaning was performed by using the same procedure as the cleaning of the seed harvested from the glasshouse.

**Table 2-1** List of all serradella accessions with source, flowering date, days to maturity and weight of seed harvested

| Accession number | Cultivar | Source | Flowering date[1] | Days to maturity | Harvested seed (g) [2] |
|---|---|---|---|---|---|
| *Ornithopus sativus* | | | | | |
| W246 | | Germany | 28/10 | 53 | 51.9/5 |
| W248 | Maia | Portugal | -- | -- | 38.1/5 |
| W249 | Aza | Portugal | 6/11 | 50 | 23.8/4 |
| W253 | Warta | " | 2/11 | 50 | 27.7/5 |
| W255 | Vinar | " | 4/11 | 54 | 39.5/5 |
| W256 | Biata | " | 3/11 | 57 | 34.1/5 |
| W341 | | England | 16/11 | 40*[3] | 12.5/5 |
| W349 | | Germany | 18/11 | 41* | 18.7/5 |
| W359 | | Spain | 4/11 | 57 | 32.0/5 |
| W360 | | E.Germany | 7/11 | 48 | 20.6/4 |
| W361 | | " | 4/11 | 57 | 53.5/5 |
| W362 | | " | 12/11 | 53 | 20.6/5 |
| W363 | | " | 10/11 | 54 | 21.4/5 |
| W364 | Gelb II | " | 3/11 | 52 | 23.1/5 |
| W365 | | " | 9/11 | 50 | 23.0/5 |
| W366 | | " | 7/11 | 52 | 31.7/5 |
| W367 | | " | 1/11 | 54 | 39.2/5 |
| W370 | | Czech. | 2/11 | 56 | 28.1/5 |
| W372 | Koha | NZ | 6/11 | 48 | 19.4/4 |
| W379 | | Australia | 30/10 | 54 | 48.5/5 |
| W418 | | SW.Europe | --[4] | -- | 12.5/5 |
| W419 | | " | -- | -- | 40.7/5 |
| W422 | | " | -- | -- | -- |
| W423 | | " | -- | -- | -- |

| Accession number | Cultivar | Source | Flowering date | Days to maturity | Harvested seed (g) |
|---|---|---|---|---|---|
| W426 | | " | -- | -- | 26.8/3 |
| WT1 | Tetraploid | NZ | 30/10 | 56 | 24.6/5 |
| WT16 | " | NZ | 3/11 | 56 | -- |
| W369 | | Czech. | 29/10 | 54 | 28.1/5 |
| W383 | | SW.Europe | 15/10 | 70 | 60.7/5 |
| O. compressus | | | | | |
| W429 | | SW.Europe | -- | -- | 36.6/5 |
| W20 | | NZ. | 8/10 | 78 | 37.5/5 |
| W22 | | NZ. | 9/10 | 74 | 40.4/5 |
| W44 | Pitman | NZ. | 3/10 | -- | -- |
| W47 | | Australia | 4/10 | 78 | 42.8/3 |
| W241 | | NZ. | 3/10 | 80 | 44.0/5 |
| W339 | | England | 20/10 | 65 | 12.4/4 |
| W340 | | " | 27/9 | 85 | 16.4/4 |
| W343 | | Italy | 26/10 | 59 | 20.8/5 |
| W346 | | " | 24/10 | 67 | 13.1/5 |
| W347 | | " | 27/10 | 60 | 12.9/5 |
| W348 | | Germany | 15/10 | 71 | 60.7/5 |
| W350 | | Portugal | 16/10 | 68 | 19.9/5 |
| W351 | | Denmark | 13/10 | 69 | 29.6/5 |
| W356 | | Portugal | 18/10 | 65 | 24.1/5 |
| W368 | | Czech. | 20/10 | 68 | 45.0/5 |
| W381 | | SW.Europe | 25/10 | 59 | 8.4/5 |
| W380 | | " | -- | -- | 46.0/2 |
| W382 | | " | 17/10 | 69 | 23.8/2 |
| W384 | | " | 19/10 | 65 | 12.3/5 |

| Accession number | Cultivar | Source | Flowering date | Days to maturity | Harvested seed (g) |
|---|---|---|---|---|---|
| W385 | | " | 27/10 | 61 | 5.1/5 |
| W386 | | " | 22/10 | 62 | 9.6/5 |
| W387 | | " | 23/10 | 60 | 9.3/5 |
| W388 | | " | 1/11 | 62 | 13.3/5 |
| W389 | | " | 28/10 | 65 | 14.4/5 |
| W390 | | SW.Europe | 27/10 | 65 | 14.6/5 |
| W391 | | " | 29/19 | 63 | 15.6/5 |
| W392 | | " | 27/10 | 62 | 13.0/5 |
| W393 | | " | 2/11 | 52* | 9.3/5 |
| W394 | | SW.Europe | 27/10 | 62 | 17.9/5 |
| W395 | | SW.Europe | 22/10 | 66 | 16.2/5 |
| W396 | | " | 23/10 | 66 | 71.0/5 |
| W397 | | " | 1/11 | -- | -- |
| W398 | | " | 27/10 | 65 | 50.4/5 |
| W399 | | " | 3/11 | 62 | 39.3/5 |
| W400 | | " | 30/10 | 54* | 57.8/5 |
| W401 | | " | 22/10 | 60 | 40.2/4 |
| W402 | | " | 29/10 | 60 | 25.8/2 |
| W403 | | " | -- | -- | 39.0/4 |
| W432 | Madeira | Australia | 8/10 | 74 | 21.4/4 |
| WT2 | Tetraploid | NZ | 2/10 | 83 | 34.0/4 |
| WT5 | " | NZ | 28/9 | 89 | 53.3/5 |
| W413 | | SW.Europe | -- | -- | 23.1/5 |
| W433 | Tauro | Australia | 10/10 | 74 | 22.7/5 |
| W434 | Avila | " | 22/10 | 61 | 68.3/5 |
| W435 | Eneabba | " | 5/10 | 78 | 15.0/2 |
| W436 | Elgarra | " | 20/10 | 62 | 30.7/5 |

| Accession number | Cultivar | Source | Flowering date | Days to maturity | Harvested s e e d ( g ) |
|---|---|---|---|---|---|
| W438 | Uniserro | " | 16/10 | 67 | 26.6/4 |
| W439 | Madeira | " | 8/10 | 75 | 31.7/5 |
| O. perpusillus | | | | | |
| W420 | | SW.Europe | -- | -- | 12.1/5 |
| W421 | | " | -- | -- | 31.4/6 |
| W424 | | " | 28/10 | 54 | 18.1/5 |
| W425 | | " | 5/11 | 48 | 23.7/5 |
| W377 | | NZ. | 28/10 | 54 | 33.7/5 |
| W404 | | SW.Europe | 29/10 | 53 | 43.6/4 |
| W405 | | " | -- | -- | 31.7/6 |
| W406 | | " | 2/11 | 50 | 16.3/5 |
| W407 | | " | 2/11 | 50 | 20.4/5 |
| W408 | | " | 2/11 | 50 | 20.9/4 |
| W427 | | " | 3/11 | 49 | 30.3/4 |
| W428 | | " | 26/10 | 56 | 20.4/5 |
| O. pinnatus | | | | | |
| W42 | | NZ. | 27/10 | 59 | 64.0/5 |
| W355 | | Portugal | 26/10 | 64 | 18.6/5 |
| W378 | | NZ. | 29/10 | 57 | 68.4/4 |
| W409 | | SW.Europe | 22/10 | 68 | 48.6/5 |
| W411 | | " | 8/11 | 49* | 49.3/3 |
| W412 | | " | 10/11 | 46* | 59.6/5 |
| W410 | | " | 24/10 | 62 | 32.7/5 |
| W414 | | " | 21/10 | 66 | 33.3/5 |
| W415 | | " | 27/10 | 59 | 30.3/5 |

| Accession number | Cultivar | Source | Flowering date | Days to maturity | Harvested seed (g) |
|---|---|---|---|---|---|
| W416 | | " | 24/10 | 62 | 41.8/5 |
| W417 | | " | 2/11 | 59 | 57.6/5 |
| W431 | | Australia | 22/10 | 71 | 34.5/5 |
| WT3 | Tetraploid | NZ | 2/11 | 59 | 32.6/5 |
| W374 | | Australia | 4/11 | 59 | 79.6/5 |
| W437 | Jebala | " | 20/10 | 66 | 26.8/5 |
| *O. sativus* x *O. compressus* | | | | | |
| W376 | | NZ. | 26/10 | 57 | 41.4/5 |
| W430 | G20 | NZ | 2/11 | 54 | 56.3/5 |

Note:
1. Flowering date was the date when the first fully open flower appeared in the field in 1990.
2. The seeds were mostly from 5 plants (amount/5) of each accession in the glasshouse in 1990.
3. The symbol '*' means the accessions matured in a shorter period than the other accessions within the species ($P < 0.05$).
4. The symbol '--' means plants died.

As heritable properties, seed characteristics are usually used to distinguish differences between accessions. In serradella, the most common trait is the percentage of hardseed. Wild materials with low percentage of hardseed would be of potential agronomic value. Segments from 22 accessions were weighed and germinability and hardseededness were measured in the laboratory at the Seed Technology Centre, Massey University. The selected samples which were harvested from the field, in

general were those from which the original seeds had been collected from a wild environment and the quantity of segments harvested was such that material was available to carry out these tests. Four replicates of twenty five segments were used to test for germinability and hardseededness, using the top of paper method at 20°C (ISTA. 1985). Before the germination

test, four replicates of fifty segments were counted, weighed, and multiplied by 20 to give 1000 segments weight. The mean was then recorded to four decimal places.

## 2.2. Experiment 2. Seed development

An experiment which followed seed development of serradella was carried out in a field at the Frewin's block, Massey University (40° 23'S, 175° 37'E). Soil type was a Tokomaru silt loam. The soil analysis was pH - 5.8, Olsen P -13, Exch K - 0.32, SO4 - 5.8, (see Appendix 1, Tabora, 1991). Following cereal in the previous year, the whole block was ploughed in March 1990 and fallowed over the winter. In spring, it was harrowed twice to prepare a seed-bed before seeding. Two plots of 2 m x 16 m were set out, 1.5 m apart, then each plot was further divided into three plots of 2 m x 5 m (separated by 0.5 m of bare ground) for three replicates of each cultivar. Two cultivars of serradella, 'Grasslands Koha' (Ornithopus sativus), and a 'hybrid' serradella (O. compressus x O. sativus) were sown on 17 September, 1990. The seed was drilled using a cone seeder at 10 kg/ha, 1 cm deep. Row spacing was 30 cm. Both samples were dehulled and the 'hybrid' because of its hardseededness, was scarified with an Electric Seed Scarifier (Westinghouse, USA, RPM 1425) for one minute before sowing. Three hand weedings were carried out during the growing season. No fertiliser, herbicides or fungicides were used.

The plants of both cultivars first flowered on 16 December, 1990. Ten days after the first flowers appeared, tagging of flowers began. This was continued weekly from 28 December, 1990 to 25 January, 1991. At each date, at least 90 (3 x 30) inflorescences were tagged with coloured wire, using a different colour for each date. All tagged pods were harvested on 11 February. 1991, so that the pod ages were 16,22,27,32,38 and 46 days respectively after first flowering.

Fresh and dry weights of 1000 segments from pods from each of the six age groups were then determined by drying the samples in the oven (105°C) in two hours, the moisture content

of segments recorded, and germination (top of paper method using 4 replicates of 25 of fresh and dried segments, after segments had been dried in the laboratory (RH 78%, 24° C) for one month), hardseededness and viability (seeds were soaked in tetrazolium solution at 35°C for 2 hours before examination) assessed. All tests followed the rules of the International Seed Testing Association (ISTA 1985).

### 2.3. Experiment 3. Hardseed breaking

Two lines from serradella species which normally are expected to have a high percentage of hard seed: 'W432' (Ornithopus compressus) and 'W431' (O. pinnatus) were chosen as experimental lots for assessing methods of breaking hard seed. Concentrated sulphuric acid (98%) was used to soak seed samples for different times ( 5, 10, 15, 20, 30, 40, 50, 60 minutes ). Firstly, one hundred seeds (dehulled with a rugose rubber board) were immersed in 5 ml sulphuric acid in small vials labelled with the different soaking times on them. After the seeds had been soaked for the prescribed time, they were washed in running water for 30 min. Subsequently, the germination test was performed using 4 replicates of 25 seeds and the top of paper method. Parallel control germination tests were set using unsoaked seed.

### 2.4. Statistical analysis

Data were statistically analyzed on the computer using a SAS programme at DSIR Grasslands. Analyses of variance were performed on the results of each test to compare differences between accessions, differences between individual accessions and population means within species for days to maturity and seed characteristics among the accessions of the same species drawn from part of the wild material in experiment 1. LSD values presented are at the 5% level. Also, the Duncan's multiple range test was used to determine significance of differences. An association between flowering date and days to maturity was determined using correlation analysis. Also, analyses of variance were used to compare the effects of seed age on seed development parameters, and the effects of hardseed breaking treatments in experiment 2 and 3 respectively.

# CHAPTER 3

# RESULTS

## 3.1. Identification and evaluation.

All of the accessions were identified as members of four species and one subspecies by means of a key to the species of Ornithopus L. adapted from 'Flora Europeae' (Plates 3-1-1 to 3-1-4). The species and subspecies were:

1. Ornithopus sativus Brot. (28 accessions, 2 hybrid lines included)
2. Ornithopus sativus Brot. subsp. isthmocarpus (Cosson) Dostal (1 accession)
3. Ornithopus compressus L. (49 accessions)
4. Ornithopus pinnatus (Miller) Druce. (15 accessions)
5. Ornithopus perpusillus L. (12 accessions)

The hybrids (O. sativus x O. compressus) in the accessions were grouped with O. sativus. The morphological and agronomic traits are described in Tables 3-1-1 to 3-1-4 and 3-1-5 to 3-1-8 respectively.

In forage crop, usually some botanical properties such as adult leaf length, leaflet number, plant height, stem length and pod length are strongly linked to agronomic performance and dry matter yield. In these four species, variation among accessions for measurements of the morphological characteristics mentioned above showed mostly continuous distributions, especially those for adult leaf length and leaflet number. However some broad discontinuous distributions occurred mainly for stem length and plant height. As examples, the distribution for adult leaf length, leaflet number, pod length, stem length and plant height of O. compressus, the most common and promising species, are given in Figure 3-1-1 to 3-1-5.

At the stage of first flower appearance, plant height of most accessions in O. compressus averaged 110 mm but ranged from 80 to 220 mm. They exhibited the prostrate growth typical of the species, except for a few accessions which were semi-erect, such as the Australian cultivars 'Avila', 'Tauro', 'Elgarra' and 'Madeira'. W403 which had been collected at very dry sites (alt. 800 & 1000 m) in Spain had the greatest plant height at 220 mm (double the mean height of the species). In addition, stem length data were also more variable than data for other traits within the four species. For example, in O. compressus stem length ranged from 120 to 440 mm, with an average of 220 mm. Accessions W380, W390 had stem lengths of more than 400 mm and 440 mm respectively. Within O. sativus, accessions such as W248, W366 and W419 had stem lengths of more than 500 mm (mean around 260 mm).

Most accessions had flower colours typical of the species they belonged to, but some of them differed e.g. O. sativus normally has white or pink flowers, but purple flowers occurred in accessions W362 and W363, and light-yellow flowers in accessions W246 and W366. Accession W428 of O. perpusillus was yellow in colour, when flower colour for the species is normally white or pink.

All 107 serradella accessions tested are listed in Table 2-1 along with their DSIR Grasslands numbers and sources. Those without cultivar names were collected or introduced from other countries. The dates when the first flower fully opened, and the days to maturity were recorded (Table 2-1) as agronomic characters of each accession grown in the field. The total amount of seed harvested from 5 plants or less than 5 plants in the glasshouse is also recorded in the same table.

There was a significant relationship between flowering date and days to maturity. The days from appearance of the first flower to seed maturity (harvesting date) decreased as first flower emergence was delayed. An analysis of all accesseions within a species indicated a negtive correlation (Table 3-1-9).

**Plate 3-1-1a.** Ornithopus sativus, stem with flowers and pods.

**Plate 3-1-1b.** Growth habit of Ornithopus sativus:
erect growth in the field.

**Plate 3-1-2a.** Ornithopus compressus, stem with flowers and pods.

**Plate 3-1-2b.** Growth habit of Ornithopus compressus:
prostrate growth in the field.

**Plate 3-1-3.** Ornithopus perpusillus and its growth habit. Above: stem with flowers and pods; below: prostrate growth in the field.

**Plate 3-1-4.** Ornithopus pinnatus and its growth habit. Above: stem with flowers and pods; below: prostrate growth in the field.

**Plate 3-1-5.** The pods of serradella species, from left to right: O. sativus, O. compressus, O. perpusillus and O. pinnatus.

**Plate 3-1-6.** The elongated, oval, spherical nodules on the roots of Ornithopus.

Figure 3-1-1. Frequency distribution of adult leaf length for 0. compressus accessions

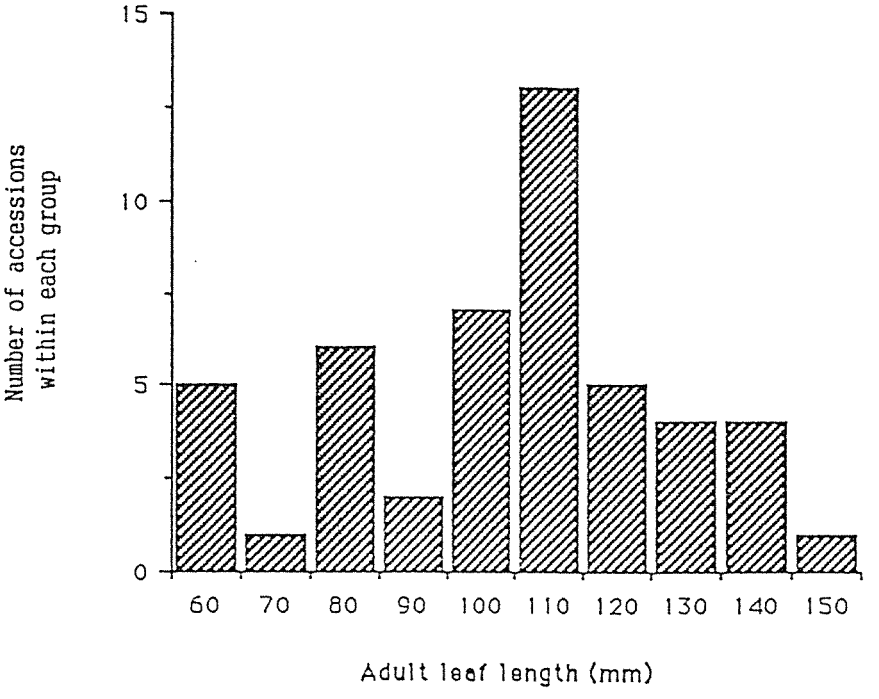



Figure 3-1-2. Frequency distribution of leaflet number for 0. compressus accessions

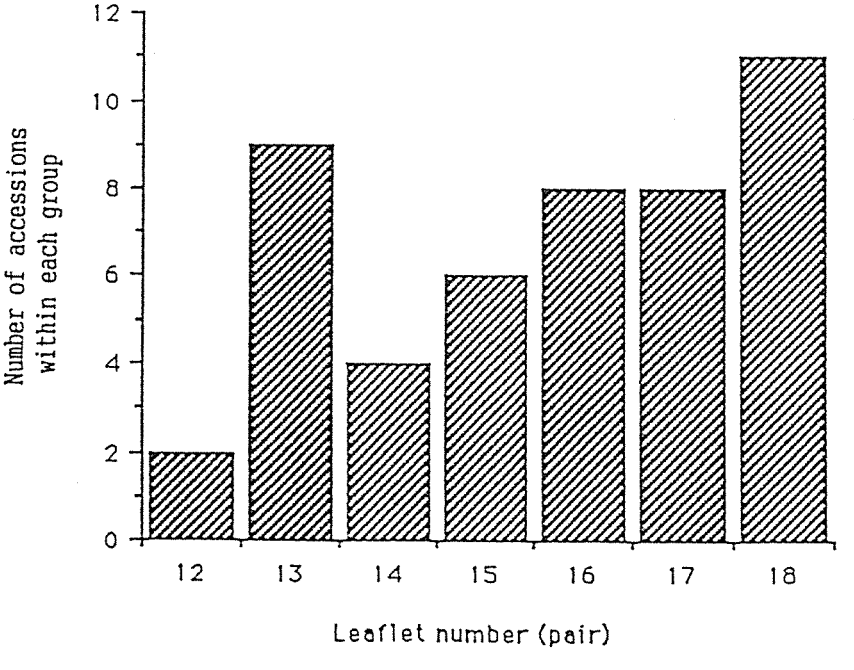


Figure 3-1-3. Frequency distribution of pod length for O. compressus accessions

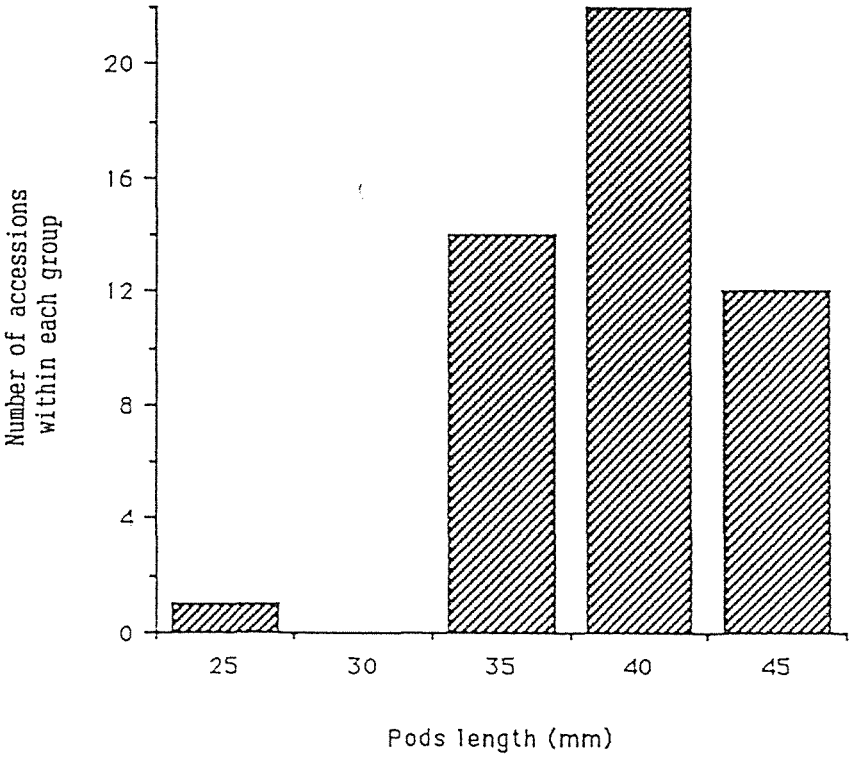


Figure 3-1-4. Frequency distribution of plant height for Q. compressus accessions

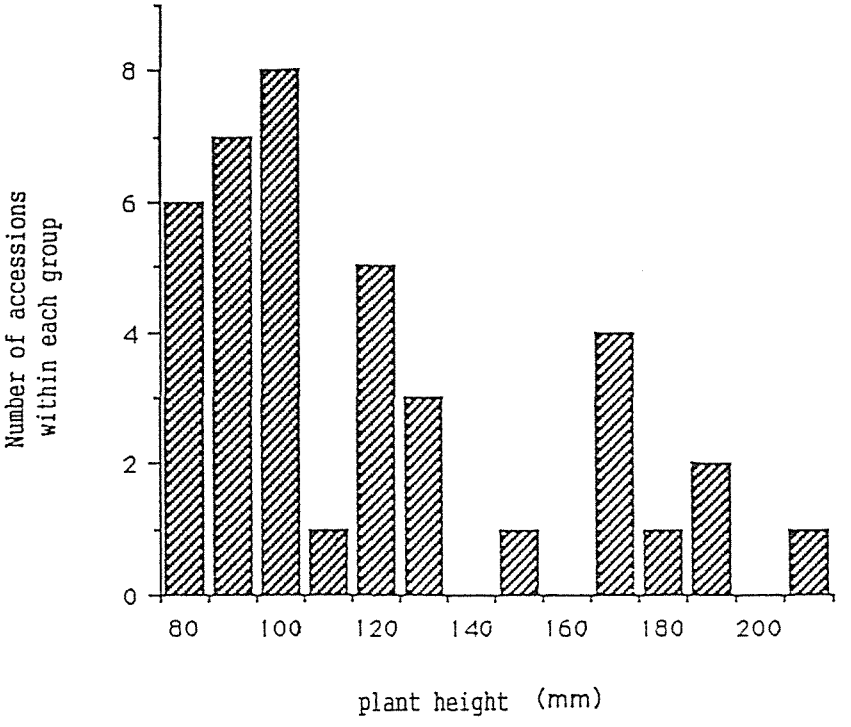



Figure 3-1-5. Frequency distribution of stem length for Q. compressus accessions

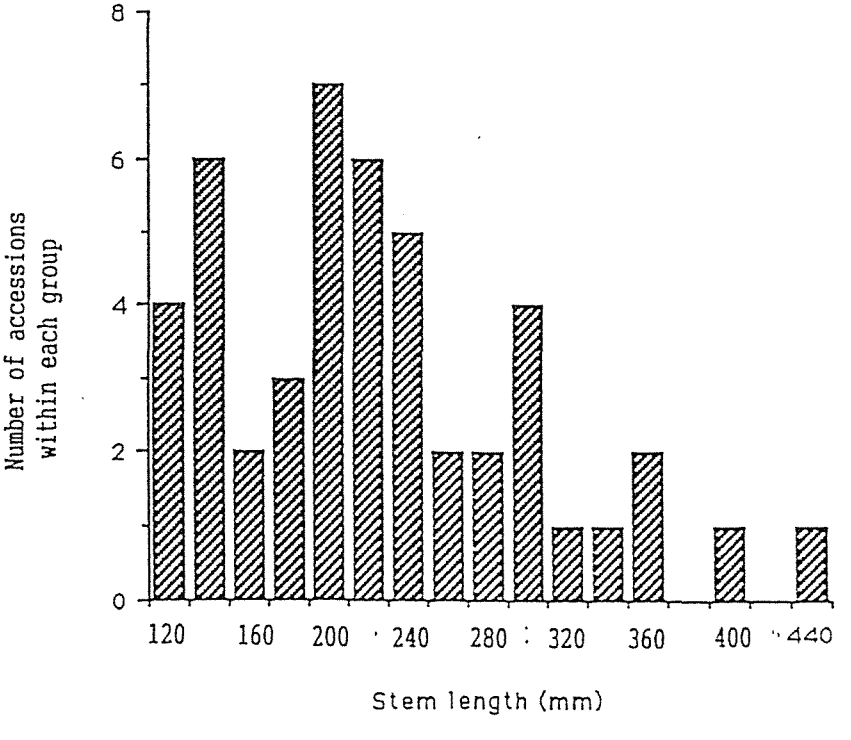


Table 3-1-1. Morphological description of Ornithopus sativus Brot. and accessions identified as belonging to this species.

| | |
|---|---|
| 1. Name of Species : | Ornithopus sativus Brot. ( Pink serradella ) |
| 2. Life Form : | pubescent annual |
| 3. Adult leaf : | 80-160 mm long (mean 127 mm), leaflets 15-20 pairs (mean 18 pairs), lanceolate (9)✡, mixture of lanceolate and elliptical (4), elliptical (13) to ovate (2) |
| 4. Plant height : | 140-320 mm (mean 250 mm) at first flower bloom |
| 5. Stem length : | 170-550 mm (mean 325 mm) at first flower bloom |
| 6. Inflorescences : | axillary head with 3-6 flowers |
| 7. Peduncle : | 30-80 mm long (mean 63 mm) |
| 8. Bract : | 10-30 mm long, with 4-8 pairs of leaflets |
| 9. Flowers : | white (7), pink-white (17), yellow-white (2) or purple (2), 6-9 mm long |
| 10. Legumes : | 2-2.5 x 15-40 mm, compressed, contracted between segments; segments 3-7, gray-yellow (Plate 3-1-5) |
| 11. Seeds : | flattened and kidney-like, dark brown |
| 12. ACCESSIONS IDENTIFIED : | W246. W248. W249. W253. W255. W256. W341. W349. W359. W360. W361. W362. W363. W364. W365. W366. W367. W370. W372. W379. W418. W419. W426. WT1. WT16. W369#. W376*. W430*. W383. W422. W423. |

Note :

✡ = refers to number of accessions with this characteristic.

# = O. sativus Brot. subsp. isthmocarpus (Cosson) Dostal.

* = Hybrid (O. sativus x O. compressus)

Table 3-1-2. Morphological description of Ornithopus compressus L. and accessions identified as belonging to this species.

| | |
|---|---|
| **1. Name of Species :** | Ornithopus compressus L. ( Yellow serradella ) |
| **2. Life Form :** | pubescent annual |
| **3. Adult Leaf :** | 60-150 mm long (mean 107 mm), leaflets 12-18 pairs (mean 15 pairs), oblong (11), oblong-elliptical (8), elliptical (14), oblong-lanceolate (2), ovate (5) and oblong-ovate (7) |
| **4. Plant height :** | 80-220 mm (mean 108 mm) at first flower bloom |
| **5. Stem length :** | 120-440 mm (mean 236 mm) at first flower bloom |
| **6. Inflorescences :** | axillary head with 3-5 flowers |
| **7. Peduncle :** | (20)* 30-60 (70,130) mm long (mean 45 mm), some peduncles of no. W399 extended above bracts but this was an uncommon phenomenon |
| **8. Bract :** | 5-5.5 mm long with 5-12 pairs of leaflets |
| **9. Flowers :** | All yellow, 5-8 mm |
| **10. Legumes :** | 15-45 mm long,curved, compressed, slightly contracted between segments; segments 2-14, elliptical-oblong (Plate 3-1-5) |
| **11. Seeds :** | flattened and kidney-like, yellow |
| **12. ACCESSIONS IDENTIFIED :** | W429, W20, W22, W44, W47, W241, W339, W340, W343, W346, W347, W348, W350, 351, W356, W368, W381, W380, W382, W384, W385, W386, W387, W388, W389, W390, W391, W392, W393, W394, W395, W396, W397, W398, W399, W400, W401, W402, W403, W432, WT2, WT5, W413, W435, W434, W433, W436 W438, W439. |

Note: * minimum length of peduncle.

Table 3-1-3. Morphological description of Ornithopus perpusillus L. and accessions identified as belonging to this species.

| | |
|---|---|
| 1. Name of Species : | Ornithopus perpusillus L.<br>( Common birdsfoot ) |
| 2. Life Form : | pubescent annual |
| 3. Adult Leaf : | 60-140 mm long (mean 85 mm), leaflets 12-18 pairs (mean 14 pairs), a tiny leaflet appeared under some normal leaflets on the plant of no. W424, elliptical (11 accessions) |
| 4. Plant height : | 30-100 mm (mean 64 mm) at first flower bloom |
| 5. Stem length : | 60-180 mm (mean 130 mm) at first flower bloom |
| 6. Inflorescences : | axillary head with 3-6 flowers |
| 7. Peduncle : | 15-30 mm long (mean 27 mm) |
| 8. Bract : | 5-20 mm long with 3-8 pairs leaflets |
| 9. Flowers : | white-pink (10), yellow (1 No. W428), 3-5 mm |
| 10. Legumes : | 1.5-2 x 100-280 mm, straight, compressed, contracted between segments, segments 4-9, elliptical-oblong (Plate 3-1-5) |
| 11. Seeds : | oblong-ovate, yellow |
| 12. ACCESSIONS IDENTIFIED : | W420, W421, W424, W425, W377, W404, W405, W406, W407, W408, W427, W428. |

Table 3-1-4. Morphological description of Ornithopus pinnatus (Miller) Druce and accessions identified as belonging to this species.

| | |
|---|---|
| 1. Name of Species : | Ornithopus pinnatus (Miller) Druce<br>( Slender serradella ) |
| 2. Life Form : | glabrous annual |
| 3. Adult leaf : | 60-120 mm long (mean 91 mm), leaflets 7-9 pairs (mean 8 pairs), oblanceolate (13) or elliptical (12) |
| 4. Plant height : | 40-120 mm (mean 69 mm) at first flower bloom |
| 5. Stem length : | 120-300 mm (mean 230 mm) at first flower bloom |
| 6. Inflorescences : | axillary head with 1-7 flowers |
| 7. Peduncle : | (30) 50-90 (100) mm long (mean 68 mm) |
| 8. Bract : | no |
| 9. Flowers : | all yellow, 6-8 mm |
| 10. Legumes : | 25-35 mm long, curved, not contracted between segments; segments 5-14, cylindrical (Plate 3-1-5) |
| 11. Seeds : | oblong (cylindrical), yellow |
| 12. ACCESSIONS IDENTIFIED : | W42, W355, W378, W409, W410, W411, W412, W414, W415, W416, W417, W431, WT3, W374, W437. |

**Table 3-1-5.** Agronomic characters for accessions of Ornithopus sativus Brot.

| | |
|---|---|
| **1. Name of Species:** | Ornithopus sativus Brot.<br>( Pink serradella ) |
| **2. Growth Habit:** | All erect |
| **3. Stem Density:** | # dense:<br>W418, W419, W426, W376<br># a few stems:<br>W246, W248, W249, W253, W255, W256, W341, W349, W359, W360, W361, W362, W363, W364, W365, W366, W367, W370, W372, W379, WT1. W383, W369, W430. |
| **4. Heads/branch:** | 7.4 (ranging from 6-12) |
| **5.*Flowering Date:** | 4/11 (in field, ranging from 28/10-18/11),<br>10/10 (in glasshouse). |
| **6. Flowering Period:** | 30-40 days |
| **7. Days to Maturity:** | Around 52 d after first flower bloom (range 40-57 days Table 2-1)<br>Matured in significantly shorter period (40 days):<br>W341, W349 |
| **8. Nodulation:** | Very good. Most nodules located on first root near to root collar, less on secondary roots (Plate 3-1-6). |
| **9. Pests and Diseases:** | None found |

Note:

\# very dense = >50 stems/plant.
dense = 10-50 stems/plant.
a few stems = <10 stems/plant.

* date first flower appeared.

Table 3-1-6. Agronomic characters for accessions of Ornithopus compressus L.

| | |
|---|---|
| **1. Name of Species:** | Ornithopus compressus L.<br>( Yellow serradella ) |
| **2. Growth Habit:** | Prostrate or semi-prostrate<br>All were prostrate except W356, W403, W432, W435, W434, W433, W436, W438, W439 which were semi-prostrate. |
| **3. Stem Density:** | # very dense: W346, W380, W382, W389, W392, W393, W394, W399, W400, W402, W413.<br>dense: W429, W20, W22, W47, W241, W339, W340, W343, W347, W350, W368, W381, W385, W387, W388, W390, W391, W395, W396, W397, W398, W401, W403, W435, W434, W438.<br>a few stems: W348, W351, W356, W384, W386, W432, WT2, WT5, W433, W436, W439. |
| **4. Heads/branch:** | 6.3 (ranging from 5-8) |
| **5. Flowering Date:** | 19/10 (in field, ranging from 27/9-3/11),<br>1/10 (in glasshouse) |
| **6. Flowering Period:** | 40-60 days |
| **7. Days to Maturity:** | Around 69 d after first flower bloom (range 52-89 days Table 2-1).<br>Matured in significantly shorter period (below 55 d): W393, W400<br>Matured in significantly longer period (over 81-90 d): W340, WT2, WT5 |
| **8. Nodulation:** | Very good. Most nodules located on first root near to root collar, but less on secondary root (Plate 3-1-6). |
| **9. Pests and Diseases:** | None found |

Note: # see note of Table 3-1-5.

Table 3-1-7. Agronomic characters for accessions of Ornithopus perpusillus L.

| | |
|---|---|
| **1. Name of Species:** | Ornithopus perpusillus L. ( Common birdsfoot ) |
| **2. Growth Habit:** | semi-prostrate |
| **3. Stem Density:** | all very dense |
| **4. Heads/branch:** | 5.3 (ranging from 4-8) |
| **5. Flowering Date:** | 30/10 (in field, ranging from 26/10-5/11), 16/10 (in glasshouse) |
| **6. Flowering Period:** | 30-35 days |
| **7. Days to Maturity:** | around 52 d after first flower bloom (range 48-56 days Table 2-1). |
| **8. Nodulation:** | Very good. Most nodules located on the first root near to the root collar with less on the secondary roots (Plate 3-1-6). |
| **9. Pests and Diseases:** | None found |

Table 3-1-8. Agronomic characters for accessions of Ornithopus pinnatus (Miller) Druce.

| | |
|---|---|
| **1. Name of Species:** | Ornithopus pinnatus (Miller) Druce.<br>( Slender serradella ) |
| **2. Growth Habit:** | All prostrate |
| **3. Stem Density:** | All very dense |
| **4. Heads/branch:** | 4.2 (ranging from 3-6) |
| **5. Flowering Date:** | 28/10 (in field, ranging from 20/10-8/11),<br>10/10 (in glasshouse) |
| **6. Flowering Period:** | 30-40 days |
| **7. Days to Maturity:** | Around 62 d after first flower bloom (range 46-71 days Table 2-1).<br>Matured in a significantly shorter period (46 d): W412<br>Significantly later maturity (62 days or later):<br>W355, W409, W410, W414, W416, W431, W437 |
| **8. Nodulation:** | Very good. Most nodules located on the first root near to the root collar with less on secondary roots (Plate 3-1-6). |
| **9. Pests and Diseases:** | None found |

**Table 3-1-9.** Correlation between flowering date and days to maturity in the four species.

| Species | Correlation coefficient (r) | Level of significance |
|---|---|---|
| O. sativus | -0.76 | 0.0001 |
| O. compressus | -0.88 | 0.0001 |
| O. pinnatus | -0.83 | 0.0001 |
| O. perpusillus | -0.94 | 0.0001 |

**Table 3-1-10.** Correlation between flowering date and leaf length

| Species | Correlation coefficient (r) | Level of significance |
|---|---|---|
| O. sativus | 0.15 | 0.33 |
| O. compressus | 0.36 | 0.0008 |
| O. pinnatus | -0.0087 | 0.97 |
| O. perpusillus | -0.09 | 0.72 |

There was no correlation between flowering date and leaf length in O. sativus, O. pinnatus and O. perpusillus, but the relationship was low but significant in O. compressus (Table 3-1-10).

Among accessions of O. sativus, W341 and W349 required significantly fewer days to maturity (38 and 41 d) compared with the species mean (52 d); W393 and W400 of O. compressus, and W411 and W412 of O. pinnatus also were shorter in days to maturity than the mean of the species they belonged to (Table 3-1-11). The accessions mentioned above matured in a significantly shorter time. For O. perpusillus, the accessions did not differ from each other in days to maturity.

**Table 3-1-11.** Comparisons between species mean and individual accessions which had a shorter number of days to maturity.

| Species | Mean of days to maturity (d) | Level of significance | LSD (0.05) |
|---|---|---|---|
| *O. sativus | 53a | 0.0001 | 7.5 |
| W341 | 38b | | |
| W349 | 41b | | |
| *O. compressus | 71a | 0.0002 | 14.5 |
| W381 | 62ab | | |
| W387 | 61ab | | |
| W343 | 61ab | | |
| W402 | 61ab | | |
| W401 | 61ab | | |
| W347 | 60ab | | |
| W400 | 55 b | | |
| W393 | 52 b | | |
| #O. perpusillus | 52 | 0.0595 | n.s |
| W428 | 57 | | |
| W377 | 56 | | |
| W424 | 55 | | |
| W404 | 54 | | |
| W408 | 50 | | |
| W407 | 50 | | |
| W406 | 49 | | |
| W427 | 49 | | |
| W425 | 46 | | |
| *O. pinnatus | 64a | 0.0001 | 13.7 |
| W411 | 49 b | | |
| W412 | 46 b | | |

'*' species mean

'#' means for all accessions when compared to each other in O. perpusillus

**Table 3-1-12.** Comparison of seed characteristics in some accessions originally collected from wild environments

| Accessions | 1000 segment weight (g) | Germination (%) | Abnormal (%) | Hardseed (%) | Fresh ungerminated (%) |
|---|---|---|---|---|---|
| *O. sativus* subsp. isthmocarpus | | | | | |
| W369 | 3.37 | 95 | 2 | 2±1 | 0 |
| *O. compressus* | | | | | |
| W385 | 5.069 f* | 59 b | 1 | 12 j | 23# |
| W390 | 4.847 g | 51 c | 2 | 19 ij | 20 |
| W393 | 4.069 h | 66 a | 2 | 16 ij | 14 |
| W387 | 4.233 h | 37 d | 3 | 24 i | 24 |
| W396 | 5.101 f | 33 d | 0 | 39 h | 23 |
| W386 | 5.697 d | 24 e | 0 | 65 fg | 10 |
| W401 | 6.822 b | 20 ef | 3 | 67 fg | 2 |
| W395 | 5.245 ef | 21 e | 0 | 73 ef | 5 |
| W400 | 5.314 e | 7 hi | 0 | 72 ef | 17 |
| W388 | 6.064 c | 5 hi | 0 | 77 de | 17 |
| W397 | 4.710 g | 9 g | 2 | 80 de | 9 |
| W394 | 4.658 g | 15 fg | 1 | 81 cd | 3 |
| W399 | 5.565 d | 1 i | 0 | 88 bc | 9 |
| W398 | 5.675 d | 0 i | 1 | 91 ab | 8 |
| W392 | 5.997 c | 4 i | 0 | 95 a | 1 |
| W402 | 7.152 a | 3 i | 0 | 97 a | 0 |
| P | 0.0001 | 0.0001 | | 0.0001 | |
| LSD (5%) | 0.204 | 7.776 | | 8.2084 | |

| | | | | | |
|---|---|---|---|---|---|
| O. pinnatus | | | | | |
| W409 | 1.07 b | 40 a | 4 | 56 c | 0 |
| W417 | 0.99 c | 15 b | 0 | 85 b | 0 |
| W412 | 0.92 d | 8 bc | 1 | 91 b | 0 |
| W411 | 1.15 a | 1 c | 1 | 97 a | 1 |
| P | 0.0001 | 0.0001 | | 0.0001 | |
| LSD (5%) | 0.034 | 8.1348 | | 7.5312 | |
| O. perpusillus | | | | | |
| W408 | 1.38 b | 75 a | 4 | 15 b | 2 |
| W424 | 1.17 c | 57 b | 8 | 25 b | 9 |
| W428 | 1.05 d | 19 c | 0 | 71 a | 10 |
| W406 | 1.85 a | 16 c | 1 | 80 a | 3 |
| P | 0.0001 | 0.0001 | | 0.0001 | |
| LSD (5%) | 0.064 | 8.964 | | 11.006 | |

Note:

* For each test, the data followed by the same letter within any column are not significantly different at the 5% level of probability.

# The percentage except germination, abnormal, hardseed and fresh ungerminated is dead seeds or empty sigmemts.

**Table 3-1-13.** Comparison between the species mean and individual accessions for hardseededness and germination within species

| Accession | Mean (H)* (%) | LSD (0.05) | Pr. | Mean (G)* (%) | LSD (0.05) | Pr. |
|---|---|---|---|---|---|---|
| +O. compressus | 78.7 a | 7.7 | 0.0001 | 11.3 e | 13.7 | 0.0001 |
| W393 | 20.0 c | | | 66.7 a | | |
| W385 | 12.0 c | | | 58.7 ab | | |
| W390 | 18.7 c | | | 50.7 bc | | |
| W387 | 24.0 b | | | 37.3 cd | | |
| W396 | 38.7 b | | | 33.3 d | | |
| +O. pinnatus | 91.6 a | 9.4 | 0.0001 | 8.0 b | 9.6 | 0.0001 |
| W409 | 56.0 b | | | 40.0 a | | |
| +O. perpusillus | 78.0 a | 9.8 | 0.0001 | 18.0 c | 8.1 | 0.0001 |
| W408 | 14.7 c | | | 74.7 a | | |
| W424 | 25.3 b | | | 57.3 b | | |

Note:

'*' Mean (H) is the mean of percentage hardseed;
Mean (G) is the mean of percentage germination.

'+' species mean.

**Table 3-1-14.** Particulars of distribution of accessions with low and high percentage hardseed in O. compressus, O. pinnatus and O. perpusillus.

| Accessions | Hardseed (%) | Altitude (m) | Habitat | site of collection | country of collection |
|---|---|---|---|---|---|
| O. compressus | | | | | |
| W385 | 12 | 100-200 | roadside | Cercal | Portugal |
| W390 | 19 | 400 | roadside | Formosa | Portugal |
| W393 | 16 | 100-200 | cork oak plantation | Montargil | Portugal |
| W387 | 24 | 200 | wet depression in parkland | St.Margarida de Serra | Portugal |
| W396 | 39 | 100-300 | wasteland | Amarante | Portugal |
| W397 | 80 | --- | sparsly grassed terrace | Amarante | Portugal |
| W394 | 81 | 10 | abandoned field | Vila Franca de Xira | Portugal |
| W399 | 88 | 600-800 | grazed ryeg-white clover paddock | Vila Pouca | Portugal |
| W398 | 91 | 400 | field | Candemil | Portugal |
| W392 | 95 | 100 | dry bank | Ponte de Sor | Portugal |
| W402 | 97 | 500 | grassland | Tuoro | Italy |
| O. pinnatus | | | | | |
| W409 | 56 | 650 | logged ecalyptus woodland | Monchique | Portugal |
| W417 | 85 | --- | uncut meadow | Santiago | Spain |
| W412 | 91 | 100-200 | rolling country | Cercal | Portugal |
| W411 | 97 | 100-200 | rolling country | Cercal | Portugal |
| O. perpusillus | | | | | |
| W408 | 15 | 750 | rocky bank | Mondenado | Spain |
| W424 | 25 | 850 | roadside | St. Eulalia | Portugal |
| W428 | 71 | 650 | logged ecalyptus woodland | Monchique | Portugal |
| W406 | 80 | 800-1000 | very dry site | Asturianos | Spain |

Some accessions originally collected from wild environments were compared for 1000 segment weight, germinability and hardseededness in a multiple comparison among individuals in the population. An interesting result was that some accessions which expressed a low percentage of hardseed came from species which usually have high levels of hardseeds, e.g. W385, W390, W393, W387 and W396 in O. compressus; W409 in O. pinnatus; and W408 and W424 in O. perpusillus (Table 3-1-12). These accessions had high germination. Also, significant differences occurred between individuals and the population for hardseededness e.g. W385, W387, W390, W393, W396 and the other accessions in O. compressus; W409 and the other accessions in O. pinnatus and W424, W408 and the other accessions in O. perpusillus (Table 3-1-13).

Particulars of original habitats of the accessions with low and high percentage hardseed are listed in Table 3-1-14 in order to detect any apparent effects of environment and geography.

### 3.2. Seed development.

In this trial pink serradella (O. sativus) cv. Grasslands Koha and hybrid serradella (O. sativus x O. compressus) were used to investigate seed development. Moisture content, viability, germination of freshly harvested seeds, germination of dried seeds and hardseededness were measured at different seed development stages (Table 3-2-1). Segment moisture content fell by only 12% in Koha and 20% in the hybrid between 16 and 38 days after peak flowering (DAPF), and did not get to under 50% during the course of the experiment. Because of limited seed numbers, it was not possible to determine actual seed moisture content. Viability increased rapidly between 16 and 22 DAPF for both species and was greater than 90% by 32 DAPF (Table 3-2-1). Freshly harvested seed was deeply dormant (0-5% germination), but air drying removed the dormancy and allowed germination to proceed (Fig. 3-2-1 and 3-2-2). Data from Table 3-2-1 infer that germination of air dried seed was higher than viability at 16 and 22 DAPF but these conflicting results are considered to be

because of sampling error, limited seed numbers, and difficulty in interpreting the tetrazolium test results in young seeds. Hard seed did not occur in Koha. In the hybrid hard seed was present until 28 DAPF, but did not occur after this time (Fig. 3-2-2).

**Table 3-2-1.** Effects of different seed ages on seed moisture content, viability, germination in freshly harvested seeds and germination in dried seed in 'Koha' and 'Hybrid'

| Days after peak flowering | Koha | | | | Hybrid | | | |
|---|---|---|---|---|---|---|---|---|
| | * SMC. | Viab. | Germ.1 | Germ.2 | SMC. | Viab. | Germ.1 | Germ.2 |
| 16 | 74.6a | 10d | 0 | 25c# | 72.8a | 14d | 0 | 37c# |
| 22 | 68.1b | 70c | 0 | 98ab# | 68.4b | 77c | 1 | 73ab |
| 28 | 65.0b | 86b | 0 | 93ab# | 61.8c | 83bc | 2 | 73ab |
| 32 | 63.4b | 96a | 1 | 100a | 63.1c | 93ab | 4 | 95a |
| 38 | 62.2c | 96a | 2 | 96ab | 59.9c | 97a | 1 | 90a |
| 46 | --- ✡ | 99a | 3 | 100a | 52.1d | 97a | 5 | 82ab |
| LSD (5%) | 5.7 | 7.7 | ns | 10.9 | 4.2 | 10.0 | ns | 33.3 |
| P | 0.0181 | 0.0001 | 0.3211 | 0.0001 | 0.0006 | 0.0001 | 0.1708 | 0.0198 |

Note:

* SMC=Segment Moisture Content, Viab.=Viability, Germ.1=Germination of freshly harvested seed. Germ.2=Germination of dried seed.

# See text.

✡ Not enough segments for testing

In any column, figures followed by the same letter do not differ significantly at the 5% level of probability in accordance with the Duncans Test.

Figure 3-2-1. Effects of seed age on viability and germination of freshly harvested `Koha' and `Hybrid' seed.

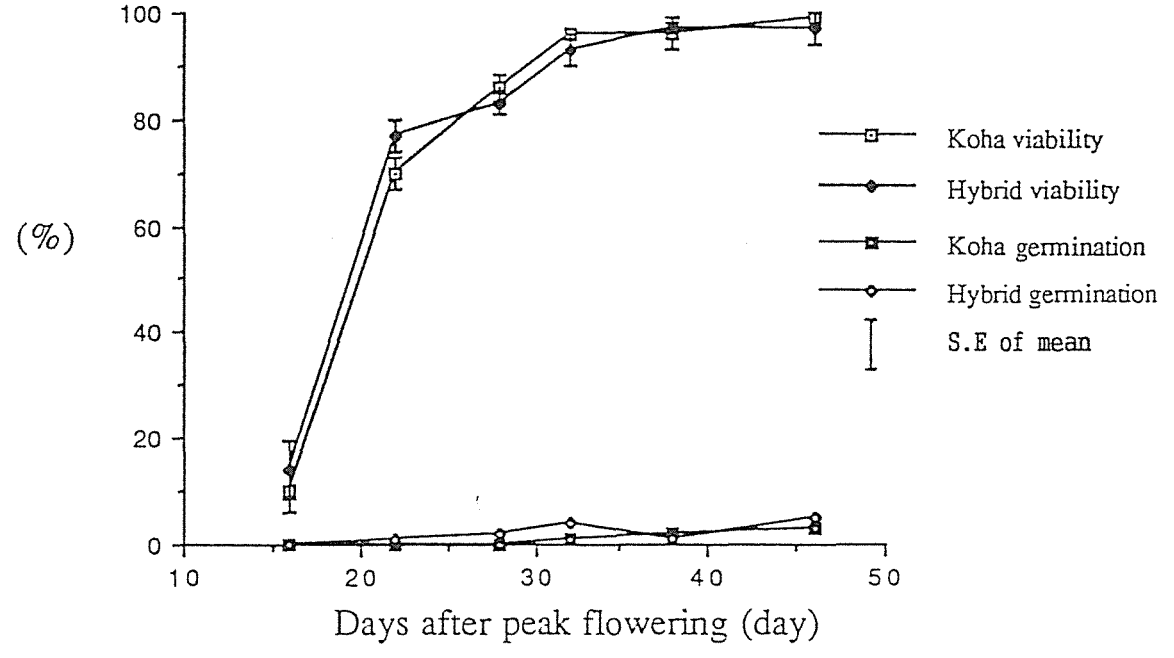
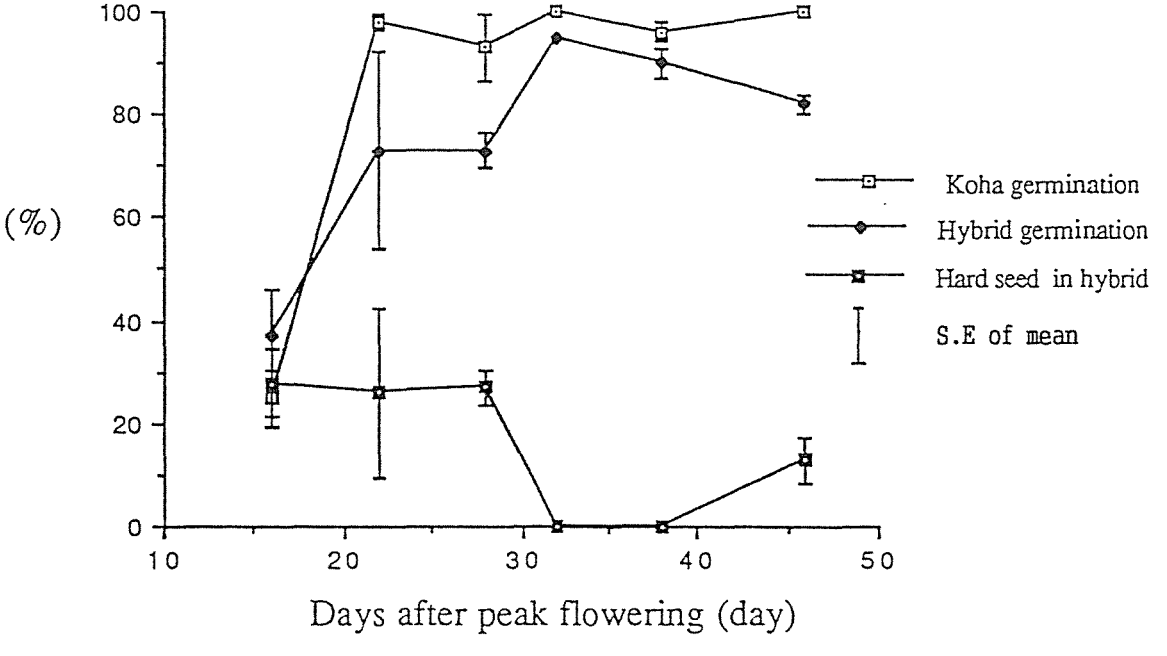



Figure 3-2-2. Effects of seed age on germination of air dried `Koha' and `Hybrid' seed, and hard seed of the `Hybrid'



### 3.3. Hardseed breaking.

Breaking of hardseed was carried out on two species, O. compressus (accession W432) and O. pinnatus (accession W431) by immersing seed in concentrated sulphuric acid for different lengths of time. The effects of the treatments on the percentage hardseed, germination, dormant seeds (fresh ungerminated seed) and abnormal seedlings are presented in Tables 3-3-1 to 3-3-3.

In O. compressus, percentage hard seed declined significantly with each 5 minutes of immersion time in sulphuric acid until after 30 minutes only 1% of seeds were hard (Table 3-3-1, Fig. 3-3-1). However it then took another 20 minutes to completely eliminate hard seed. In O. pinnatus, hard seed was initially reduced after 5 minutes, but no further reduction occurred until after 20 minutes (Fig. 3-3-2) and no treatment completely eliminated hard seed.

In both species, 90% viability was recorded after 30 minutes immersion, and viability did not significantly increase with further immersion time. Thirty minutes was also the time at which germination was greatest in O. pinnatus, although for O. compressus, germination did not differ between 30 and 40 minutes (Table 3-3-2). However in both species a secondary dormancy was induced. This increased with increasing immersion time in O. compressus and tended to do the same in O. pinnatus. However, in O. pinnatus, the treatment also induced abnormal seedling development (Table 3-3-3), particularly after more than 30 minutes of immersion, whereas in O. compressus, abnormal seedlings did not develop.

After the treatment which produced the greatest germination in the two species i.e. 30 minutes immersion, the two significantly differed in germination, dormant seed and abnormal seedlings, but there was no difference in the percentage of hardseed and viability (Table 3-3-4).

**Table 3-3-1.** Effect of time of immersion in sulphuric acid on hardseed (%) of two serradella species

| Treatment | Hardseed (%) | | |
|---|---|---|---|
| (min.) | O. compressus | O. pinnatus | LSD (5%) |
| 0 | 88 b | 68 a | 18.7 |
| 5 | 66 c | 45 b | 14.1 |
| 10 | 52 d | 48 b | 20.1 |
| 15 | 29 e | 47 b | 18.4 |
| 20 | 20 f | 28 c | 15.6 |
| 30 | 1 g | 11 d | 14.1 |
| 40 | 1 g | 15 d | 8.2 |
| 50 | 0 g | 9 d | 11.6 |
| 60 | 0 g | 5 d | 3.2 |
| P | 0.0001 | 0.0001 | |
| LSD (5%) | 8.2 | 11.8 | |

**Table 3-3-2.** Effect of time of immersion in sulphuric acid on germination (%) and dormancy (%) of two serradella species.

| Treatment (min.) | O. compressus | | | O. pinnatus | | |
|---|---|---|---|---|---|---|
| | Germ.(%) | Dorm.(%)■ | Viab.(%)✡ | Germ.(%) | Dorm.(%)■ | Viab.(%)✡ |
| 0 | 2 e | 10 f | 12 | 11 f | 21 ab | 33 |
| 5 | 8 de | 26 e | 34 | 47 bc | 8 cd | 55 |
| 10 | 15 cde | 31 de | 46 | 44 bcd | 7 cd | 51 |
| 15 | 29 bc | 41 cd | 70 | 37 cde | 13 bc | 50 |
| 20 | 23 cd | 54 bc | 77 | 52 ab | 16 bc | 68 |
| 30 | 40 a | 51 c | 91 | 61 a | 23 ab | 90* |
| 40 | 51 a | 44 cd | 95 | 34 de | 27 a | 83* |
| 50 | 29 bc | 67 ab | 96 | 36 cde | 30 a | 86* |
| 60 | 30 bc | 69 a | 99 | 45 bcd | 21 ab | 91* |
| P | 0.0001 | 0.0001 | | 0.0001 | 0.0001 | |
| LSD (5%) | 15.4 | 14.1 | | 12.5 | 10.6 | |

* These figures include abnormal seedlings.

■ secondary dormancy --- seeds imbibed, but not germinated (ISTA 1985).

✡ excluding hard seeds (i.e. Germ. + Dorm %)

**Table 3-3-3.** Effect of time of immersion in sulphuric acid on abnormal seedling (%) of two serradella species.

| Treatment | Abnormal seedling (%) | | |
|---|---|---|---|
| (min.) | O. compressus | O. pinnatus | LSD (5%) |
| 0 | 0 | 0 b | 0 |
| 5 | 0 | 0 b | 0 |
| 10 | 0 | 1 b | 3.2 |
| 15 | 0 | 3 b | 3.2 |
| 20 | 3 | 2 b | 3.2 |
| 30 | 0 | 6 b | 3.7 |
| 40 | 3 | 22 a | 18.3 |
| 50 | 0 | 20 a | 12.7 |
| 60 | 1 | 25 a | 17.2 |
| P | 0.0959 | 0.0001 | |
| LSD | ns | 7.9 | |

**Table 3-3-4.** Comparison of two species for percentage germination, hardseed, dormant seed, abnormal seedlings and viability after 30 minutes immersion in sulphuric acid.

| | Observations | | | | |
|---|---|---|---|---|---|
| Species | * Germ.(%) | Hard.(%) | Dorm.(%) | Abn.(%) | Viab.(%) |
| O. compressus | 40b | 1 | 51a | 0b | 91a |
| O. pinnatus | 61a | 11 | 23b | 6a | 90a |
| P | 0.0427 | 0.0892 | 0.0293 | 0.0138 | ___ |
| LSD (5%) | 19.7 | ns | 22.7 | 3.7 | ___ |

* Germination; Hardseed; Dormant seed; Abnormal seedlings and Viability.

Figure 3-3-1. Effects of sulphuric acid treatment on hardseed and germination performance in O. compressus

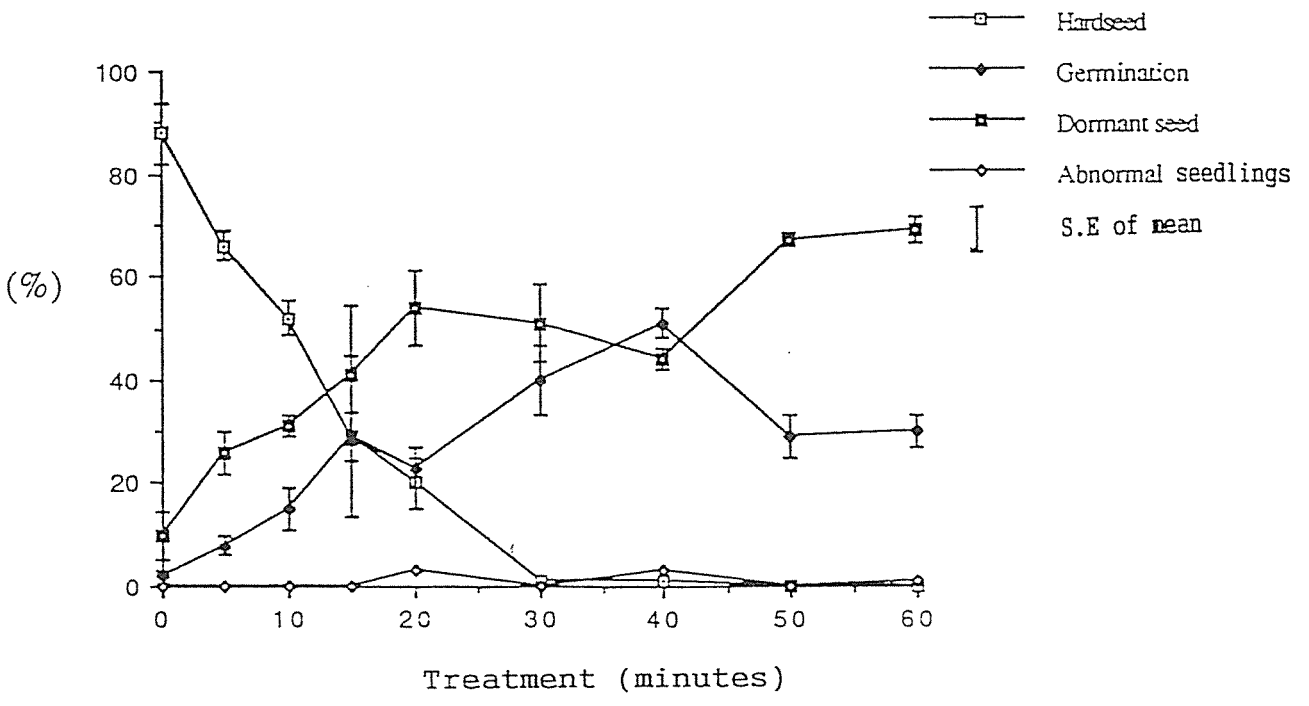
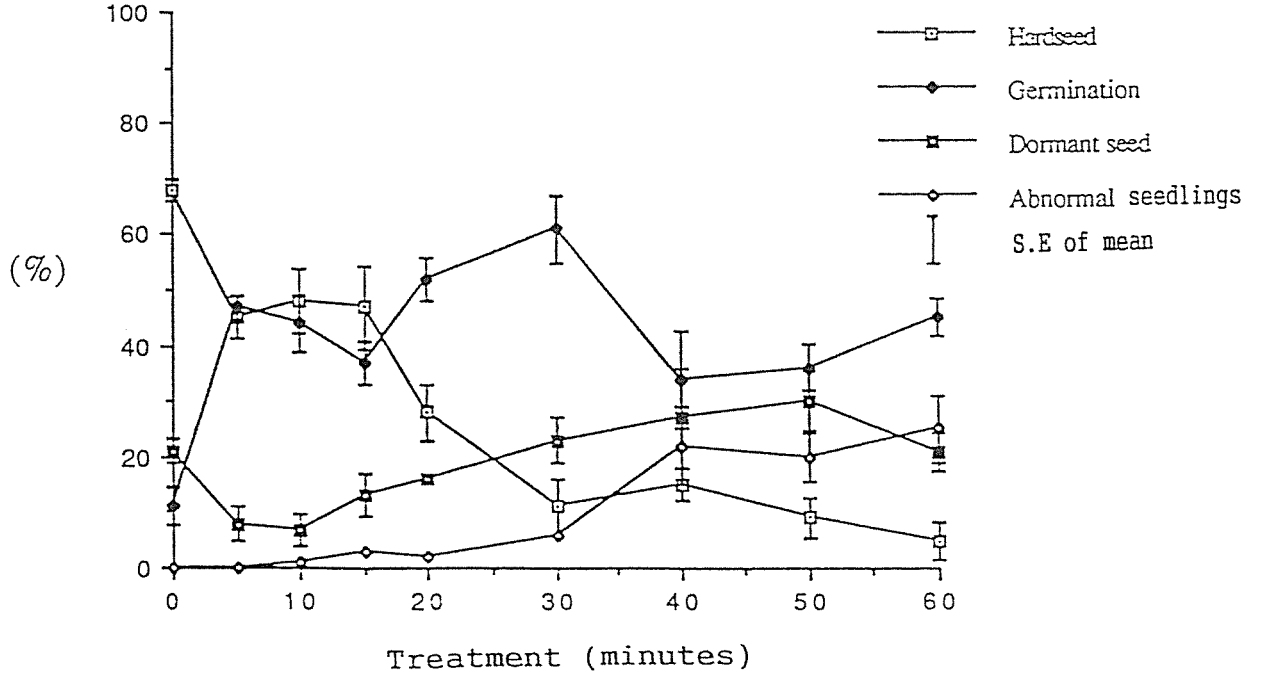



Figure 3-3-2. Effects of sulphuric acid treatment on hardseed and germination performance in O. pinnatus.



# CHAPTER 4

# DISCUSSION

In this study accessions were described, identified and then grouped into four species and one subspecies. Distinguishing them did not present major difficulties as the genus consists of only six species and subspecies (Polhill et. al., 1981). Botanists and taxonomists have previously differed in the total number of species considered to be in the genus; for example, 10 species (Willis, 1973) and 15 species (Allen, 1981), but documentary evidence and descriptions of species have been provided only for O. sativus, O. sativus subsp. isthmocarpus, O. compressus, O. pinnatus, O. perpusillus and O. micranthus. Until such time as other evidence is provided, only these six species are considered to be involved in the genus.

Four species recorded and described in 'Flora Europeae', (O. sativus, O. compressus, O. perpusillus and O. pinnatus) were collected from wild environments from Portugal (40 accessions), Spain (9 accessions) and Italy (1 accession) during a DSIR collecting trip in 1986. The subspecies O. sativus ssp. isthmocarpus was introduced from Czechoslovakia to New Zealand in 1987. In accordance with the frequency of collection, it appears that Portugal is rich as a serradella resource (40 of the total 50 accessions came from Portugal). Whether other Mediterranean countries are also as valuable sources of serradella is not known because the data provided did not state whether this was a truly random collection or concentrated only on collection in special areas. Other introduced accessions (Table 2-1) were also included in the study.

Frankel (1947, 1989) suggested that the characters of concern to plant breeders were broadly speaking, of two kinds: (a) observable or strongly expressed characters

(morphological, physiological or biochemical characters relating to survival, productivity or quality) which can be identified in single plants or their immediate progenies; (b) variable or complex characters, which are subject to environmental variation and are largely responsible for differences in yield or adaptation. This study emphasized the first kind of characters which in fact, are thought to be of basic importance (Mochrie et. al., 1981; Wills, 1983 and Rys et. al., 1988).

Most of the accessions showed morphological characteristics typical of the species to which they belonged. However, a number of accessions within species did differ in some morphological properties from those regarded as normal for the species. Usually, these were variations in flower colour. Accessions W362 and W363 in O. sativus had completely purple and purple flowers with white-purple wings respectively, whereas the normal colour is white or pink. A bright yellow flower colour appeared in accessions W246 and W366. These accessions all came from Germany. Accession W428 of O. perpusillus had yellow flowers (normal colour is white or pink), and was collected from a wild environment in Monchique, Portugal. Usually, changed flower colour in the natural state is a variation of the species that is easily noticed during collection. Accession W424 in O. perpusillus produced a variant leaf consisting of a whole leaf with single or a pair of much smaller leaflets under some normal leaflets. These appeared on nearly every single leaf of the plant. So far its heritability has not been determined.

Accession W369 had morphological characters typical of O. sativus subsp. isthmocarpus (Tutin et. al., 1968). The pods usually had a long, narrow, cylindrical constriction between the segments, the beak was more than 10 mm long and curved, but a germination test revealed a percentage hard seed of 2% (Table 3-1-12). In a study of hard seed in this subspecies carried out by Bolland (1985), a proportion of over 90% of hardseed was reported. The difference from present results may result from variation among ecotypes in the subspecies.

Within O. compressus, it was apparent that flower colour and size, adult leaves, leaflet number, peduncle length and legume length were the least variable characters and showed continuous distributions (Fig. 3-1-1 to 3-1-3). By contrast, more variable characteristics were plant height and length which expressed discontinuous distributions. Most accessions in the species fell within the height range of 80-140 mm. However accessions W439, W387, W388, W432, W433, W434, W436, W356 and W403 were taller (in the range of 170-220 mm). Most of these accessions are cultivated varieties e.g. W432, W433, W434, W436, W439 from Australia, W356 from Portugal, and their growth habit has no doubt been "improved" through selection and breeding. This improved growth habit is suitable for hay production and is able to increase biomass on the ground by using space more effectively. Some wild accessions such as W387, W388 and W403 (220 mm high) also revealed the same property. These were collected from wet depressions in a parkland, from a hay paddock and from a very dry site in Portugal and Spain respectively. They are ecotypes of the species which have evolved in particular environments, and are regarded as having a trait which could be useful for improving the usual prostrate habit of O. compressus for hay production. On the other hand, an advantageous trait is sometimes relative, and is also dependent upon its purpose of use. For example, O. compressus is often valued for its prostrate growth habit which gives it improved tolerance to grazing.

Although the stems of most yellow serradella accessions grew to a length of around 220 mm, some had extended to 380 mm by the time of first flower appearance. W390 and W380 had the longest stems of 400 and 440 mm respectively. Growth differences among varieties or wild material are important as longer stems in general result from superior growth ability or vigour of the plant itself. This is a heritable characteristic and selection for it should result in improved forage production, as it eventually contributes to more rapid accumulation of biomass in weight/area and over
time.

Unlike O. pinnatus and O. perpusillus, O. sativus does not normally have a large number

of stems. However among the tested wild accessions, W418, W419 and W426 did produce plants with many stems, and therefore possess a strong branching ability. Accession W419 also had the longest stems (more than 540 mm) and good seed production (8.14g/plant), compared with other accessions in this group (mean 6.2g/plant). In terms of the traits mentioned above, it is regarded as possessing potential for strong vigorous growth.

Annual forages complete a whole life cycle in a specific period within a year if the environment is suitable for them to grow. The serradella species differed from each other for different stages of growth and while some exhibited early flowering, others were very late in moving from the vegetative to the reproductive state. For example with flowering, O. sativus first flowered around 4 November at Palmerston North (Appendix 2), O. compressus around 19 October, O. perpusillus around 30 October, and O. pinnatus around 28 October. However the reproductive cycle differed among the species as they all had very similar dates of seed maturity (pink serradella 26/12, yellow serradella 27/12, common birdsfoot 21/12 and slender serradella 29/12). Several accessions of yellow serradella were early bloomers, but matured late. This might be linked to special requirements for temperature and light during growth and development, as the best material will show the closest adaptation to the temperature and light regimes available. Flowering date was correlated with days to maturity in serradella, in that the days from appearance of the first flower to seed maturity decreased as first flower emergence was delayed. Correlative levels differed from each other, but correlations were usually very high in all species except O. sativus.

The correlation analysis for flowering date and leaf length did not show clearly whether there was a positive or negative relationship, mainly because the number of samples used was limited. Although, O. compressus (with a larger sample number compared with the other 3 species) expressed a positive correlation, i.e. leaf length increased as flowering date was delayed and the difference was significant (Table 3-1-10), this was not strong enough to certify that the performance would be likely to occur in the other species in the genus. It could be useful to confirm this in future work.

For dry matter and seed production, annual forages which have a long vegetative period, and a short reproductive period based on completing a whole life cycle, are of interest. Differences for these characters occurred not only between species, [for example flowering date was 4 November with 52 days to maturity in O. sativus; 19 October with 69 days to maturity in O. compressus; 30 October with 52 days to maturity in O. perpusillus and 28 October with 62 days to maturity in O. pinnatus (detailed in Tables 3-1-5 to 3-1-8)], but also within species. For example in O. sativus, accessions W341 and W349 matured in 38 and 41 days after the first flower bloomed respectively. They were longer (by 10-14 days) in the vegetative phase than other accessions. Also, this was a significant reduction in days to maturity compared with that of other accessions or with the mean for the species. These two accessions were introduced from England and Germany and possibly are cultivated varieties. Similar results were recorded in O. compressus and O. pinnatus as well. Two yellow serradella accessions, W400 and W393, and accessions W411 and W412 of O. pinnatus were shorter in days to maturity than the mean of the species. They were collected in Portugal, the former two from different environments (a water-logged terrace (alt. 500 & 1000 m) and a cork oak plantation (alt. 100 & 200 m)) respectively. The latter two were collected from the same site, rolling country (alt. 100 & 200 m) and might belong to the same ecotype.

All of the accessions which matured in a short period had a similar trait; that is they bloomed late and ripened two weeks quicker than others within the species. Quick maturity after flowering is considered to be a very useful agronomic property for dry matter production and seed production (Humphreys, 1978). A long flowering period is undesirable if serradella is used for grazing animals as a relatively long vegetative growth phase is required. If harvesting seed after grazing, a period of vegetative recovery is more favourable to the plant. In addition, a late flowering habit and quick ripening is most suitable for harvesting quality seed and decreasing seed loss during the seed development and maturation period (Hampton, 1990).

All accessions tested in this study successfully produced seed in an unheated glasshouse

(Table 2-1), except for accessions W362, W363 (poor seed set), W422, W423 and WT16 (failed to survive) of pink serradella, W44 (failed to survive) and W397 (flowered but no seed set) of yellow serradella. The seed multiplications outside were good in the accessions of O. sativus but not satisfactory in some accessions of O. compressus, O. pinnatus and O. perpussilus. This could have been caused by the influence of too much rainfall and a poorly-drained unsuitable soil type. Most successful stands of serradella are grown in areas with an annual rainfall of about 500 mm, and on well-drained slightly acid to very acid soils (Freebairn, 1980; Gladstones et. al., 1964; Bolland, 1985). Some seedlings of some accessions in O. compressus and O. pinnatus which survived showed normal growth but produced little seed because of poor seed setting. Because of an inadequate number of seedlings to transplant into the field, or because seedlings died early following transplanting, it was not possible to get a complete observation and study of seed production.

Some of the accessions originally collected from wild environments were compared for 1000 segment weight, germinability and the percentage of hardseed. In O. compressus, accessions W402, W401, W388 and W392 possessed a high 1000 segment weight which differed significantly compared with other accessions (Table 3-1-12). Often there is a positive relationship between seed size and seedling field performance, in that large seed is considered more vigorous than smaller seed within an accession. There is also evidence that seed size differences between accessions of the same species can affect performance (Stickler et. al., 1963; Taylor, 1972; Smith et. al. 1975; Haskins et. al., 1975; Smittle et. al., 1977; McKersie et. al., 1981), but this is not always true. For example Wang (1989) showed that smaller seed of red clover was of higher vigour than large seed when two seed lots were compared. Although the seedlings of these accessions mentioned above did not show big differences in seedling performance during a germination test this could not be taken as evidence that potential vigour differences might not exist. However, accessions W386 and W387 which were not as heavy as the four accessions listed above, expressed strong and vigorous seedling growth and were regarded as the accessions with a potential for high seedling vigour.

Among the tested accessions collected from wild environments, W385, W390, W393, W387 and W396 of yellow serradella differed significantly from other accessions in the percentage of hardseed. Similarly, W409 of O. pinnatus and W408 and W424 of O. perpusillus, (compared with other accessions within the species), were also significantly lower in percentages of hardseed.

Hardseed is considered to be a problem mainly in yellow serradella and slender serradella which are the more commonly used species for hay production and grazing purposes (Barrett-Lennard et. al., 1964). Hard seed can be caused by both genetic and environmental factors. The difference between the completely soft seed of O. sativus and the high percentage hardseed of O. compressus signifies the existence of genetic variation (interspecific). However genetic differences also exist within a species e.g. the difference between accessions within O. compressus for hard seed characteristic could also have resulted from genetic differences. Spontaneous mutations are a likely source of such intraspecific variation.

Within O. compressus, accessions with a low percentage of hardseed are considered uncommon. This species usually has hardseed levels of over 90% and it was unexpected to find so big a difference between the low and high percentage hard seed. It is known that selection for adaptation to a particular habitat or niche can lead to genetic variation of properties which are heritable. In this study, not enough information on the original environments and geographic distributions of the accessions is known to be able to determine accurately the real reasons for the hardseededness variation, even if all five accessions with a low percentage of hardseed came from Portugal (Table 3-1-14). Further screening work with molecular biotechnology such as gel electrophoresis could be one method to determine if these five accessions differ in their genetic makeup compared with the other accessions.

The water impermeability of the testa of hard seed is a physical exogenous dormancy (Nikolaeva, 1969). In Western Australian serradella, a water-soluble substance present in the husk is also thought to be a germination inhibitor which passes into the seed during

imbibition (Barrett-Lennard et. al., 1964). It is suggested from this study that such a substance may possibly be present in the husk of other accessions in the species as well. The germination test on those accessions originally collected from wild environments revealed high percentages of fresh ungerminated (dormant) seed in O. compressus compared with the accessions of O. pinnatus and O. perpusillus, especially in the accessions with low hardseed contents. Germination may have been inhibited by the same or a similar substance, despite conditions considered suitable for seed germination (ISTA,1985). However, the presence of such an inhibitor was not confirmed in this study and this aspect requires further investigation.

In serradella seed development, segment moisture content decreased slowly (in 30 days) from 72.8% to 52.1% in the hybrid and similarly in O. sativus cv. Koha although the latter result was affected by use of limited segment numbers. It was not possible to obtain the actual seed moisture (Table 3-2-1), because it was extremely difficult to separate fresh pure seed from the husk to determine moisture content of seed. Moreover, it was a cloudy season with a high relative humidity (84%) during harvest, so that seed husks would have absorbed more moisture than segments harvested in a dry climate.

Seed viability of both serradella cultivars increased sharply between 16 to 22 days and reached its highest percentage at 32 days after peak flowering. The viability data were lower than the germination data for air dried seed at 16 and 22 DAPF for Koha and at 16 DAPF for the hybrid respectively in Table 3-2-1. This result probably occurred because of sampling error, (because of the small number of segments available) and difficulties with interpreting the tetrazolium test. The tetrazolium test used to determine viability should have been repeated for 16 and 22 DAPF, but no seed was available to do this.

The germinability of immature and mature seeds was extremely poor in fresh seeds of both cultivars, but it was considerably improved after drying in ambient conditions in the laboratory for one month. This suggested that desiccation is necessary for serradella seeds to switch off the process of seed development and prepare the seed for germination. In many

orthodox seeds loss of moisture during maturation is a key pre-requisite for germination (Coolbear, 1990). Such drying initiates the change from developmental metabolism to germinative growth. Action may be directly at the transcriptional level or drying may induce changes by increasing or decreasing the concentration of hormones; for example decreasing abscisic acid (ABA) concentrations, increasing gibberellin (GA) concentrations, and changing tissue sensitivity to hormones, e.g. decreasing sensitivity to ABA, or direct effects on the genome (Coolbear, 1990).

When the two cultivars were compared at the same seed age, there was little difference except for hardseed levels. There was no hardseed development in cv. 'Koha', but there was in the hybrid. Most of the hybrid parental lines had more than 70% hard seed (Williams et. al., 1987). In the seeds of the hybrid produced in this study, a hardseed level of 28% was found in seed at 16 days and 27% at 28 days, but it appeared to decrease to 0% at 32 days and then apparently jumped back up to 13% in seed at 46 days after flowering. This result was therefore not able to express the situation of hardseed development well. The reasons for the result are ascribed mainly to sampling error due to use of small seed samples. Further work should be done on this aspect in future. Although the data were not satisfactory for showing hardseed development (because the level was very variable), the evidence indicates that hardseed was apparent in the seed at 16 days after peak flowering.

Because seeds of most serradella species are difficult to separate from the segments, they can not be scarified in the normal manner to reduce the proportion of hard seeds and increase germination. Although, segments of both O. compressus and O. pinnatus were dehulled with a rugose rubber board, the percentage hardseed was still high, being 88% in O. compressus and 68% in O. pinnatus. Thirty minutes was the optimum time to treat hard seeds with concentrated sulphuric acid in both these serradella seed lots. At this point of time, nearly all the hardseed was broken, a high percentage germination was obtained, and viability of both serradellas was maintained (Table 3-3-4). However abnormal seedlings appeared in O. pinnatus following treatment for longer than 30 minutes. These abnormal seedlings were

caused by acid damage to tissue inside the seedcoat during seed immersion. Abnormal seedlings did not occur in O. compressus following the same treatment. This may result from a difference in seedcoat structure between the two serradella species, but this is not known.

Secondary dormancy occurred in untreated seeds of both serradella species (Table 3-3-2). It remained at the same level in O. pinnatus even after acid treatment for 60 min. The percentage of dormant seed should not increase if acid treatment was not a factor causing seed dormancy. In O. compressus, however, the percentage rose from 51% after 30 minutes to 69% after 60 minutes acid treatment. It is not yet clear why this occurred in this case. Further study may be useful to determine the mechanism of serradella dormancy.

Sulphuric acid treatment is a well recognized and effective method of softening impermeable seed, particularly in woody plants (Quinlivan, 1971). It is quick and does not need complex equipment. Rolston (1978) summarized some reports where a sulphuric acid soak for 30 min. was reported to break hard seed successfully in Strophostyles helvola (Hutton & Porter, 1937), Cercis canadensis (Afanasiev, 1944), Colutea istria (Koller & Negbi, 1955) and Coronilla varia (Brant et al, 1971). Acid scarified seeds showed faster field emergence and ground cover than non-scarified or hard seeds of common vetch (Aswathiah, 1987; Viado, 1989). For researchers wishing to scarify small hand harvested quantities of Lotus uliginosus seed, sulphuric acid immersion is safe and effective (Hare and Rolston, 1985). However sulphuric acid treatment can be dangerous (to the seed and operators) and the method is not suitable when large amounts of seed are treated in commercial operations. Problems of damaging seed after an inappropriate immersion technique or time can occur, as can environmental pollution caused by releasing the acid after seed treatment. Also, it is difficult to handle a large amount of sulphuric acid solution safely or store it safely for future use.

Seed of serradella treated with sulphuric acid to break hardseed is usually immersed dehulled rather than the immersion of undehulled segments. At present, obtaining pure seed

without the husks is an extremely difficult operation in yellow serradella, and therefore sulphuric acid could not be used on a commercial scale before an effective means of dehulling segments is found. Soaking segments in concentrated sulphuric acid is not effective (Fu, unpublished data) as the immersion time is longer, which increases the possibility of damaging seeds. The sulphuric acid method is therefore restricted to use for small amounts of dehulled seed in the laboratory.

Barrett-Lennard et. al. (1964) suggested at least two ways to overcome both seed impermeability and husk-imposed physiological dormancy: complete dehulling and scarification of the seeds, or exposing the pod segments to alternating high (60°C) and low (15°C) temperatures. The former usually causes much damage to seed because of the physical shape and structure of the yellow serradella pod segment, but it is a quick method for obtaining processed seeds; the latter takes time (several months) but in Western Australia is an inexpensive method using natural temperature variation in a dry climate (Barrett-Lennard et. al., 1964).

The pod segments of yellow serradella are rigid, tough and non-dehiscent. After harvesting the pod, hard seeds would develop under a natural desiccation. Seed is usually broken and damaged too much when mechanically processed. If segments are 'softened' with moisture before dehulling, the process could aid the separation of seed from the segments. However the percentage of hard seed is usually over 90% in yellow serradella, and moisture intake occurs at a different speed in the seedcoat and the pod husk. The pod husk will absorb moisture immediately in an environment with high relative humidity, but not the seedcoat. This difference in moisture uptake could possibly be used for dehulling, as the softened husk might be more easily removed. However it was not possible to test this idea in this study because of time limitations.

# CHAPTER 5

## CONCLUSION

This study is mainly a description and primary evaluation of one hundred and seven accessions of serradella. The description and record of morphological characteristics is essential work for newly collected accessions of plant species. This work should be done as soon as possible after collection in order to get useful information and have it available for use. The enhanced utilization of plant germplasm resources greatly depends on the gathering, recording and filing of new information.

Results from this study showed that morphological characters in general varied very little. However a lot of differences occurred in the agronomic characteristics among accessions within species. For example, some accessions matured in a significantly shorter period than others. For an annual forage legume, this is an interesting character when the plant is required to be used for hay production, grazing animals and seed production, or seed production after grazing in the early stages of plant growth. Differences in percentage hard seed among the accessions of wild materials occurred in O. compressus, O. pinnatus and O. perpusillus. Cultivars with a low percentage of hardseed are highly desirable, and accessions with lower hardseed levels should be used in selection or breeding programms, especially in the breeding of O. compressus. Although, some accessions with low hardseed were identified, further work will be required to determine the agronomic merit of these accessions, and to determine whether this characteristic is able to be transferred to other selections if required.

The water soluble substance reported by Barrett-Lennard et. al. (1964) or a similar substance preserved in the husks of O. compressus inhibits germination during seed imbibition. The substance occurs not only in West Australian serradella but most probably

also in accessions of O. compressus collected from different sites. Further work is required to determine effective way of removing this block to germination in this species.

Although seed yield was not an important part of the study, and reliable data were obtained from the glasshouse only, there was variation in seed production ability between accessions. A study of seed production capabilities is required as a further evaluation of these accessions. Seed yield is one of the most important indices in evaluating a forage pasture species.

Seed development can be linked to forage evaluation and seed characteristics can be a part of study of agronomic particulars. Although some work was done in this study, the results were not very satisfactory, particularly these for hard seed development. This is an extremely important aspect for serradella, and this work needs to be repeated.

Breaking hard seed with sulphuric acid is a relatively simple technique for a small amount of seed in the laboratory. Thirty minutes was the optimum time for breaking over 90% of hard seed, and retaining high viability and germinability of seed. However the development of secondary dormancy after sulphuric acid treatment is not yet understood and it would be useful to carry out a further study on seed of O. compressus. Meanwhile, several other methods such as freezing and radiation should be investigated in order to determine their effectiveness for breaking hard seed. In addition, it is also important to find a method for dehulling serradella segments. This is a key to reducing hardseededness and breaking dormancy of serradella seeds.

This study was a primary evaluation of serradella accessions available in New Zealand. As a limited contribution to knowledge it could be useful to people who are interested in forage germplasm resources or breeding pasture species. The seed multiplied during this study is available for further research on individual plant performance of serradella or a more comprehensive evaluation of the accessions.

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# GLOSSARY

**Actinomorphic:** with the parts radially symmetrical

**Apomict:** plant producing viable seed without fertilization

**Autogamous:** automatic self-pollination within a flower

**Bilabiate:** with 2 lips

**Claw:** a narrow stalk-like base of a petal or sepal

**Conduplicate:** folded together lengthwise, along the midrib, with the upper surface within; of cotyledons folded lengthwise around an incumbent radicle

**Dehiscent:** opening, usually regularly, to shed contents when ripe

**Dentate:** with sharp teeth perpendicular to margin, the sinus +/- open

**Diadelphous:** in 2 sets, as of stamens in most papilionoid Fabaceae where 9 are united and one is free

**Emarginate:** with a shallow notch at the apex

**Entire:** with a continuous margin completely lacking teeth.

**Facultative:** optional

**Gibbous:** somewhat swollen on one side, usually near the base, forming a pouch or sac

**Imbricate:** overlapping, like roof-tiles; in buds, with the edges of the organs overlapping, but not regularly

**Keel:** a sharp central ridge, like the keel of a boat; used also of the united anterior petals of papilionoid Fabaceae

**Lenticular:** the shape of a +/- circular, biconvex lens

**Limb:** the expanded part of a petal or sepal

**Oblique:** with sides unequal or meeting unequally

**Paripinnate:** evenly pinnate, i.e., without a terminal leaflet

**Pedicel:** the stalk of an individual flower

**Peduncle:** a common leafless axis bearing several flowers

**Phyllode:** a widened petiole or rachis functioning as if a blade

**Ptyxis:** (vernation) the arrangement of leaves in the bud stage

**Puberulent, Puberulous:** covered with exceedingly fine, short, dense hairs

**Pubescent:** clad in short soft hairs

**Serrate:** sharply toothed with the teeth pointing forward

**Sessile:** without a stalk

**Standard:** the upper and broad more or less erect petal of a papilionaceous flower

**Supervolute:** leaf margin rolled outside the other in the bud stage

**Terete:** circular in cross-section

**Truncate:** appearing as though cut squarely across, especially a broad square base to a leaf

**Tubercle:** a small wart-like swelling

**Tufted:** forming clumps

**Valvate, valvar:** of dehiscing fruits, opening by valves; of perianth segments in bud meeting at the edges but not overlapping

**Villous:** clad in long soft hairs not matted together

**Wing:** the lateral petals of a papilionaceous flower

**Zygomorphic:** having only one plane of symmetry; used especially of irregular flowers

(from 'Flora of New Zealand')

# APPENDIX 1

## DESCRIPTION OF THE SOIL TYPE IN THE EXPERIMENTAL SITE
(Massey University, Palmerston North, New Zealand)

TOKOMARU silt loam soil. It is classified as an aeric fragiaqualf (gleyed yellow-grey earth) and is part of the rolling country at the foot of the western Tararua ranges, New Zealand. Below is its soil analysis:

| Element | Figure Obtained | Element | Figure obtained |
|---|---|---|---|
| pH | 5.4 | Nitrate | 15.0 |
| Calcium | 515.0 | Phosphorous A | 14.3 |
| Magnesium | 3.4 | Phosphorous B | 20.0 |
| Potassium | 36.0 | Iron | 5.0 |
| Ammonium | 0.5 | Manganese | 5.0 |

Explanation of figures: pH .... measured in water, Magnesium, Potassium, Ammonium, Nitrate, Iron, Manganese.... parts per million measured in the soil extract determined according to the improved method of Morgan-Venema.

Phosphorus A — parts per million measured in Sodium Bicarbonate solution of pH 8.5 (half hour Olsen method).

Phosphorous B — parts per million soluble in diluted Sulfuric Acid (Beater method).

# APPENDIX 2

Climatological data from April 1990 to Jan. 1991

| | | Mean Temp. (Outside) (°C) | Mean Temp. (in glasshouse) (°C) | Evaporation 0.1 mm (Total Monthly) | Rainfall 0.1 mm. |
|---|---|---|---|---|---|
| 1990 | Apr. | 14.4 | 20.0 | 679 | 663 |
| | May | 11.9 | 17.8 | 399 | 921 |
| | Jun. | 9.4 | 16.7 | 201 | 1245 |
| | Jul. | 9.0 | 16.1 | 222 | 854 |
| | Aug. | 10.1 | 17.2 | 358 | 1112 |
| | Sep. | 10.1 | 18.9 | 641 | 169 |
| | Oct. | 13.4 | 20.6 | 1030 | 837 |
| | Nov. | 14.9 | 21.1 | 1152 | 983 |
| | Dec. | 14.4 | 22.2 | 1601 | 507 |
| 1991 | Jan. | 17.6 | 25.0 | 1630 | 1202 |

(from AgResearch Grasslands P.Nth.)