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Cocksfoot (*Dactylis glomerata* L.) Seed Production

**A Thesis Presented in Partial Fulfilment
of the Requirements for the
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ABSTRACT

Seed yield reduction due to variation in genotype, management, and incidence of diseases is common and well documented in grass seed crops. Two New Zealand cultivars (Grasslands Wana, Grasslands Kara) and two Japanese (Akimidori Makibamidori) cocksfoot cultivars from a three year old stand (sown in September 1991) at Grassland Aorangi Research Farm in the Manawatu were evaluated for their seed yielding capacity. In addition these cultivars were evaluated to determine the effectiveness of bactericide (Streptomycin), nematicide (Vydate/Oxamyl) or a combinations of bactericide and nematicide to determine their effect on seed yield with traditional Fungicide (Folicur) application being used as a control. The unit plot size was 1.2 X 3.0 m², with each plot containing 4 rows. A randomized block design with two factors (cultivar and pesticide) was utilized with 4 replications. In each cultivar pesticide were sprayed in four replicates from each block. The New Zealand cultivar Wana outyielded the other three cultivar and produced about 23%, 32%, and 70% higher pure seed/unit area than that cvs Kara, Makibamidori and Akimidori respectively. The ability of cv Wana to outyield other cultivar was largely due to its ability to produce a greater numbers of fertile tillers and higher number of florets/head. Seed yields in the New Zealand cultivar Kara and the Japanese cultivar Makibamidori were similar but significantly higher than cv Akimidori. The significantly higher seed yield of cv Kara was due largely to its significantly higher seed weight than the other cultivars. Similarly the higher seed yield in cv Makibamidori compared to Akimidori was mainly due to higher seed weight. The lower pure seed yield in cv Akimidori was apparently due to lack of proper photoperiodic effect or cold temperature during anthesis and its earlier seed developing resulted in more empty florets than the New Zealand cultivar and cv Makibamidori. Fertile tiller numbers spikelets/ head and florets/ head were significantly higher in cv Wana compared to the other 3 cultivars. However, the TSW was low. The TSW was highest in cv Kara. Highest recovery of pure seed was obtained from cv Wana in screen and blown fraction. Highest pure seed was obtained from cv Makibamidori in clean seed fractions. Foliar disease intensity in all the four cultivars was found to be similar at all 3 stages of plant growth although disease levels were generally low.

Percentage purity in clean seed as well as in different cleaning fractions varied depending on cultivar but cv Akimidori was always ranked lowest. Seed germination percentage in single and in multiple florets was higher in cv Akimidori compared to the other 3 cultivars.

Different cultivars reacted differently to the application of pesticide. Cultivar Wana produced the highest yield 103 g pure seed/m² in the Fungicide (Folicur) treatment compared to 55, 38 and 22 g/m² in cv. Kara, Makibamidori and Akimidori respectively. However all cultivars produced lower yields following the application of Streptomycin. Seed yield in cv Akimidori was not affected by any pesticide application. The lower yield in Streptomycin treatments was mainly due its phytotoxic effect on leaf tissue.

Seed yield in Fungicide, Vydate and in V+S treated plots was similar but significantly better than in Streptomycin treated plots. Fertile tiller numbers were also similar in these treatments but spikelet and total floret numbers were higher in the V+S treatment than in the Fungicide treatment. TSW and florets/ spikelets was not affected by pesticide application.

The recovery of pure seed was always lowest in the Streptomycin treatment in all fractions of machine cleaning of seed but was higher in V+S than Fungicide treatment, particularly in the machine clean fraction. However, the recovery of pure seed from the screen fraction did not vary significantly. Over all the foliar disease severity was low in Fungicide and V+S treatment and highest in Vydate treatment at maturity but no difference during vegetative growth or at peak flowering.

The percent purity varied only in the air screen fraction but was high in the Vydate treatment. Seed germination was high in all pesticide treatments and being above 90% in term of single florets and 85% in multiple florets with minor improvement in V+S treatment both in single and multiple florets and in Fungicide treatment with multiple florets only.

The second trial involved only one cultivar (cv Tekapo) and involved 4 plots from each block with 3 different types of fungicide Alto (cyproconazole), Bavistin (carbendazim), and Bravo (chlorothalonil) applied from flower initiation to harvest maturity at intervals of 14 to 21 day. No effect of fungicide occurred in seed yield or in yield components. Disease severity varied significantly only at harvest maturity and was lowest in Alto treated plots (2.75) compared to the control (4.25). However, no difference occurred in assessment at the vegetative stages or at peak flowering. No significant effect of fungicide was observed in terms of seed cleaning. The percentage purity was found higher in Alto and Bravo treated plots. Germination results were well above accepted levels (90%) in all fungicide treatments with Bavistin treatments being particularly useful.

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CHAPTER I

INTRODUCTION

Cocksfoot is an important perennial grass widely cultivated in temperate regions of the world. It is a stout, tussock forming plant which was brought to New Zealand in the 1840's and has a unique history of seed production initiated by the early settlers after clearing of heavy forest in the Banks Peninsula area (Christchurch) (Coulson 1979). The first seed production programme was initiated in 1852 by a farmer known as 'Cabbage' Wilson of Christchurch and spread to the Banks Peninsula area (Coulson 1979). Herbage seed production in New Zealand dates back more than 100 years with exports in the 1880s of 1400 tonnes of grass seed mainly cocksfoot (*Dactylis glomerata* L.), ryegrass (*Lolium* spp.) and tall fescue (*Festuca arundinaceae*) Mackay (1987). Cocksfoot is increasingly popular in New Zealand due to its ability to withstand dry conditions, being one of the most drought resistant temperate forage grasses (Norris & Thomas 1982; Volarie, 1991); its ability to grow in low fertility soils; its highly palatable forage production at the early growth stage; its companion ability with clover and other grasses and its ability to grow well in shade (Anon, 1965). Seed export is a major component of the New Zealand herbage seed industry totalling 15025 t in 1993 (Anon 1994). Cocksfoot is the most important New Zealand certified herbage grass seed crop after ryegrass with a total of 515 t being produced from 1000 ha in the 1994 harvest season. The two New Zealand cultivars, Grassland Wana (390 t) and Grassland Kara (97 t) made up the bulk of the seed produced, the former cultivar being bred for sheep pastures, while the latter is used mainly for dairy pastures (Rowarth *et al.*, 1991).

Although Grasslands Kara and Wana cultivars were bred under New Zealand conditions, they also have potential for use overseas, and exports of cocksfoot seed to countries such as Australia and Chile earned the New Zealand seed trade nearly \$ 600,000 in 1990. (Rowarth *et al.*, 1991). While seed exports of New Zealand cultivars continue to increase, there is the possibility of multiplication and re-export of seeds of overseas cultivars in New Zealand, particularly for the Japanese market, which uses cocksfoot as a component of dairy pastures, but does not produce seed. Seed requirements are imported primarily from USA (both US cultivars and Japanese cultivars multiplied in USA, B McCloy, pers. comm.).

In the 1992 harvest season, seed yields of both New Zealand cultivars averaged around 500 Kg/ha (MAF, 1994). Although yields of over 1000 kg/ha have been recorded (Rolston, 1991), factors influencing reduction in yields are varied but pathogens causing various diseases alone or in combination can be important. In recent years the introduction of rust resistant/tolerant cultivars like Wana and Kara has improved the performance and quality of cocksfoot but both leaf spot and seed head diseases can still be remain a serious problem, particularly in cool and wet seasons (Welty, 1989a). In New Zealand about 36 different diseases have been isolated from cocksfoot with most being due to fungal pathogens (Penycook, 1989). Among these, leaf spot disease caused by *Mastigosporium rubricosum* (Dearn. and Barth.) is considered important (Sprague, 1950; Welty, 1989a; 1991;) as well as the bacterial pathogen *Clavibacter rathayi* (Rathay's disease) recorded in Denmark (Skou, 1965) and in other countries (USA, Switzerland etc.). The causal agent of Rathay's disease or yellow slime disease (Denmark) was also first recorded in New Zealand in 1956 (Johnston, 1956). The main damage caused by this pathogen is profuse development of a yellow bacterial slime cover on the upper parts of plants, especially the inflorescence. Such parts often become dwarfed and distorted, and the inflorescence may fail to emerge from the sheath which is firmly stuck together by slime (CMI, 1973). Symptoms similar to Rathay's disease were noticed in various seed crops in growers fields at Canterbury during the 1993-94 cropping season. Preliminary investigation at Lincoln University indicated the presence of some fungal pathogens (*M. rubricosum*, *Ascochyta* sp., *A. sorgi*, *Puccinia* sp., *Stemphylium* sp.) and a bacterial pathogen *Pseudomonas syringe* (Lincoln Univ. investigation unpublished report). Similarly preliminary investigations on inflorescences from seed crops in the Manawatu and seed samples from Christchurch also suggested the presence of the fungus *M. rubricosum*, and the bacterium *Pseudomonas* spp. as well as another bacteria. The colony of this isolated bacterium appears similar to an authenticated culture of *C. rathayi* (Seed Technology Centre, Massey University). In addition some free living/ fungal and bacteria feeding, and plant parasitic nematodes were also observed in a cocksfoot seed crop at AgResearch Grasslands Experimental Station, Aorangi near Palmerston North. It has been established in wheat and in ryegrass that the nematode *Anguina tritici* can transmit *Clavibacter tritici* in wheat (Sabet, 1954) causing identical disease symptoms to those observed in cocksfoot (Sabet 1954; CMI, 1973) although it has not been established as a pathogen transmission agent in cocksfoot. Nevertheless this does suggest the possibility of nematode vector transmission of *C. rathayi* in cocksfoot (CMI, 1973). In Australia ryegrass toxicity where seeds are replaced

by nematode larvae in association with the bacterium *C. rathayi* (Bird and Stynes, 1977) which also produces toxins) can result in cattle dying after eating infected seed (Berry and Wise, 1975; Price *et al*, 1979a, Stynes and Bird, 1980; Stynes and Wise, 1980). In this case the nematode is also a species of *Anguina funesta* (Price *et al* 1979b) and the toxin bacterium is also *C. rathayi* (Stynes *et al*, (1979).

Fungicides are an effective way of controlling leaf diseases, especially when one or more fungal pathogens occur on the same plant. Their use has been studied since the early 1940s to control rust in cool-season grasses grown for seed (Hardison 1963 and 1975). More recently, however, increased seed yield, following the application of fungicide (Hampton, 1986, Rolston, *et al* 1989, Welty, 1991), has been shown to improve seed yield, herbage growth and the establishment of cocksfoot and other grasses (Clements *et al*, 1982; Spaul and Mewton, 1984 Clements *et al* 1985). Similarly Rolston *et al* (1989) has reported a 21 % increase in cocksfoot seed yield following fungicidal application (propiconazole) to cv Grasslands Wana. Although fungicides applied before or at anthesis have been shown to increase seed yield in many temperate grasses (including cocksfoot) mean commercial seed yields in New Zealand over the last few years have remained constant in major cocksfoot seed growing areas despite the application of fungicides which have been found to be effective elsewhere.

The present investigation was designed to meet three objectives:

- (1) To determine the possible role of fungicide application, bactericide use and/or the application of a nematicide in increasing cocksfoot seed yield and quality. These areas of chemical use have not been extensively studied in New Zealand and were chosen in an attempt to try to improve the unexplained low yields of cocksfoot seed experienced by many New Zealand seed producers, particularly during the 1993 and 1994 harvest seasons.
- (2) To compare the seed yield and quality of four cocksfoot cultivars two of New Zealand origin (Wana and Kara) and two from Japan (Makibamidori and Akimidori) and their reaction to chemical application.
- (3) To evaluate the effectiveness or otherwise of three different fungicides (Alto, Bavistin and Bravo) on the seed yield and yield components of cocksfoot cv Tekapo.

CHAPTER II

LITERATURE REVIEW

2.1. Origin and distribution of cocksfoot

Cocksfoot is an important temperate perennial species widely cultivated worldwide. It is native to Europe (Clapham *et al.*, (1962) and has made a significant contribution to pasture production in many temperate regions through its ability to extend the main period of green feed supply well into the summer. It is a member of the Gramineae family. The genus *Dactylis* is present in all temperate zones and according to *index kewensis* includes as many as 30 species. Most are tetraploid but there are some diploids. Cocksfoot occurs in hedgerows, meadows, roughgrass and, occasionally, in open woodland from 0-600 m above sea level, being absent only from highest parts of Scotland (Hubbard, 1954). In the northern Hemisphere it is normally found in areas bounded by the 10^o and 27^o C isotherms (Beddows, 1959), although spring frosts can injure the developing inflorescence and cause "winter burn" of the leaves (Beddows, 1959). Cocksfoot was brought to New Zealand in the very early days of the settlement by the Hay family of Pigeon Bay (Newton 1979). They used it very largely in a grass mixture for sowing down after the bush was felled and burnt. It was found to be such a good grass for stock that it soon become popular in the region, providing excellent annual fodder, especially for cattle making, vigorous growth even under drought very well condition.

2.2 Growth and reproductive development

2.2.1. Tillering

Tillering in grasses is a continuous process, and thus at any one time the grass plant comprises a population of tillers, differing in age, position on the plant and size. The tiller is the basic morphological unit, being a branch which originate from an axillary bud at the stem base. In grasses the tiller may be vegetative and barren, or may develop into a reproductive tiller bearing an inflorescence. The ratio of reproductive to vegetative tillers varies between species and varieties and within varieties and is also influenced by environment (Spedding and Diekmahns, 1972). Since tillers arise in the axil of leaves, the potential rate of tillering is dependent on the rate of leaf production. The young plant tillers profusely in autumn, late winter, and early spring, although tillering in early spring may be first, second, or third order. Under seed crop conditions viable tiller density in cocksfoot in the United Kingdom has been shown to decline from an average of 768 per 10 square decimeters on May 1 to 390 by June 15, the time of seed harvest

(Taylor and Templeton, 1966). In general, increased temperature increases the rate of tillering (Robson, 1969), and optimum temperature (about 15°C) is lower. Tillering is, however, decreased by low light intensity and increasing photoperiod (Mitchell, 1953; Ryle 1966a and b). N.P. and K all increase tillering, N showing a positive interaction with the other two elements (Langer, 1959). In cocksfoot the proportion of tillers that produce inflorescences rises with decreasing plant density (Lambert, 1963) but the proportion falls with an increase in number of tillers per plant concomitant with ageing (Lambert 1963, 1966a 1966b). Applied N has little effect on the proportion of tillers that produce inflorescences, but does increase tiller numbers and therefore numbers of inflorescences per plant. When penetration of light within the plant is increased following defoliation, the proportion of tillers producing inflorescence increases in the first year, when the number of tillers per plant is lowest. However there is less effect of identical treatment in the second year stands, and none in the third year when the number of tillers per plant is highest in cocksfoot (Lambert 1966b).

2.2.2. Floral development in cocksfoot

Floral development in cocksfoot results in an increase in the size of existing shoots or tillers and is usually associated with reduced root growth and inhibition of new tiller production as indicated by Langer (1963). Most temperate grasses require a photoperiod longer than a certain minimum for the initiation and subsequent development of the inflorescence (Evans, 1964). Although exact photoperiodic requirements vary with climatic and agronomic origin of the material (Cooper, 1963), the light intensity needed for the induction of flowering is very low, 100 lux being adequate for cocksfoot and ryegrass as described by Spedding and Diekmahns (1972). Climatic variation in flowering requirements has been reported in many grasses (Spedding and Diekmahns 1972). Even after initiation in an appropriate photoperiod, subsequent floral development and seed production are affected by light intensity and photoperiod. For example in cocksfoot and ryegrass a reduction in light intensity reduces both the number of tillers which form ears and the fertility of those which do so, mainly by reducing the number of florets on primary branches, rather than the number of primary branches themselves (Ryle, 1966c).

2.2.3. Reproduction and dry matter production for seed

Vegetative growth in cocksfoot is restricted to increases in leaf and root weight and to the expansion of new tillers. Two further highly active centres of growth, the stem and the inflorescence, are added to these at reproduction. The development of an inflorescence terminates the ability of a tiller to initiate further leaves and inflorescence growth is ensured by the expansion of already initiated but as yet unexpanded leaves on the apical meristem Speeding and Diekmahns (1972). It has also been suggested in different grasses (Hunt, 1956; Langer, 1958, Salisbury, 1965) including cocksfoot (Knight, 1965) that reproductive shoots are capable of faster growth than shoots growing vegetatively. The economic yield of a grass crop consists of a particular fraction of total dry matter production not solely on the whole photosynthetic performance of the crop. Most carbohydrate in cereals is formed from CO₂ assimilate after the ears emerge. Sugars formed before ear emergence and stored in the shoot or root contribute little (15%) to the grain (Thorne, 1966). The CO₂ absorbed after ear emergence by that part of shoot above the flag-leaf node, including the ear, accounts for most of the grain dry weight observed in barley and wheat (Thorne, 1963; 1965), which also suggests that stored sugar does not contribute much to the grain. So growth before ear emergence affects grain yields only indirectly, by affecting the potential surface available for photosynthesis after the ears emerge, and by influencing the number of potential sites at which starch can accumulate.

2.3. Environmental effect on seed yield and yield components

2.3.1. Environmental requirement for seed production

Cocksfoot generally requires a minimum annual rainfall of 600 mm and a growing season of at least eight months. It is adaptable to a fairly wide range of soil but does better on rich loams. It is better suited than ryegrass to medium moisture and fertility conditions, but will not thrive where drainage is poor. Cocksfoot grows well under shade (Anon., 1965). The development of the inflorescence in grasses is usually determined by response to local conditions of light (both duration and intensity) and temperature at each stage of development. This requirement varies greatly with species and cultivar and is related to the climatic or agronomic origin of the material (Cooper, 1960). Once the shoot is induced, the morphological change to reproductive growth generally depends

on exposure to suitable daylength, although light, light intensity and temperature may modify the response (Ryle, 1966a). Most temperate herbage grasses must pass through an initial vegetative phase during which they are insensitive to environmental conditions termed the "juvenile" stage. During this stage plants increase in size and weight but make no progress towards flowering. The duration of the juvenile stage in cocksfoot may last for only a few weeks Calder (1963). Environment plays a key role and ultimate seed production depends on the environmental condition enjoyed by the crop. For example an examination of macroenvironmental factors during anthesis of perennial ryegrasses showed that minimum screen temperature accounts for 70% of the variance observed in the number of seed/m² (Hampton and Hebblethwaite 1983a). The duration of anthesis and liberation of pollen also depends largely on weather conditions before anthesis and weather condition on the previous day respectively as indicated by Gregor (1928). Low night temperatures decrease subsequent flowering intensity and reduce the number of florets open at peak anthesis on the following day (Hill, 1971). Lambert (1966c), and Vough (1975) studied the effect of relative humidity in a wide range of grasses and concluded that it has no effect on anthesis. Rather, it is the actual presence of rain that hinders or inhibits anthesis. Wind velocity has also been shown to hinder or even inhibit anthesis in some grasses eg. in ryegrass as described by Hill (1980) although Vough (1975) found that wind speed had no effect in cocksfoot, brome grass, tall fescue, and timothy. Strong winds during the reproductive phase however can cause severe seed losses through lodging and shedding of seeds. Temperature conditions may have long term effects by affecting vegetative growth of the crop, floral induction, growth and differentiation of the inflorescence, flowering, pollen germination, seed set and seed maturation. The optimum temperature and shape of the temperature response is different for each phase and will differ between and within species (Hampton, 1994). The change from the vegetative to reproductive phase may also be modified by temperature and some species and cultivars have a critical temperature requirement as in cocksfoot, which requires vernalization. In such cases plants must be subjected to cold conditions for induction, otherwise they remain vegetative and do not become reproductive (Calder 1964a). However, exposure to high temperature may reverse the vernalized state if only partial vernalization has been achieved. For example in cocksfoot, Calder, (1964b), found short days above 10⁰ C can replace low temperature vernalization as a requisite

for subsequent flowering in long days. Cocksfoot is also one of the temperate grass species known to be particularly susceptible to late season frosts as indicated by Ede, (1968). Nikolaevskaya (1973) observed in cocksfoot that frost destroyed pollen viability and as a result no seed was formed when flowering plants were placed in frost conditions of -3 to -6°C for 3.5 to 5 hours. Lighter frosts can also influence pollen viability, delay seed development and reduce panicle numbers. In extreme conditions panicle production may fail completely as observed in cocksfoot at -10°C by Niemelainen (1989). Niemelainen (1991a) also showed in a pot trial that frost after floral initiation decreased the number of panicles, the effect being strongest at -10°C -6°C and -3°C all of which had also negative effects on panicle numbers. Panicle production in cocksfoot was more sensitive to frost injury at the late stage of development than in the early development stages, at booting for example a -7 to -9°C (soil surface) has been shown to destroy about one third of panicle in a 2nd year stand (Niemelainen, 1991a)

2.3.2. Environmental effect on flower initiation:

Once cocksfoot plants are fully induced, they can initiate inflorescence when grown in an appropriate photoperiod. Environmental conditions influence the response to photoperiod. Leopold (1951) also states that in general N level may have a profound influence on growth subsequent to floral initiation, Calder and Cooper (1961) also noticed that both nitrogen and spacing have profound effects on floral initiation in cocksfoot. Cocksfoot has been shown to be a long day plant (Tincker 1925) but before responding to long days it must be fully induced. However some workers claim plants only need an inductive requirement (Wycherley, 1952), while others show short photoperiod is necessary (Hanson and Sprague 1953), and still others suggest that both low temperature and short days are necessary. Calder (1963) therefore suggests that cocksfoot S. 143 has a juvenile stage which lasts about 5 weeks; that there is no progress to flowering in continuous light prior to induction and that continuous light before induction does not inhibit induction or post inductive development.

2.3.3. Environmental effect of on seed set

Pollination is the term used to describe the process occurring from the time of anther dehiscence until the pollen reaches the embryo sac in the ovary. Fertilization on the

other hand is the actual fusion of the male nucleus with the ovum (Hill 1980). During these process, as well as anthesis, potential yield reduction can occur. As with other grasses, cocksfoot is wind pollinated, and therefore the distance over which the pollen must travel and the duration in relation to receptivity of the stigma is important (Fairely, 1993). Although the viability of pollen may last up to 24 hours (Fairely, 1993), anthesis in grasses lasts less than 20 minutes (Langer, 1972). This means that open florets may have a short period of time in which to capture available pollen. Also, pollen does not remain viable for as long in bright sunshine (Langer 1972), which leads to desiccation and subsequent failure of fertilization. There is no continuous body of evidence to indicate which factors are important in affecting seed setting in cocksfoot. In general, environmental factors which delay or prevent the opening of florets reduce the chances of pollination as described by Ryle (1966c). Low temperature (Jones and Brown, 1951) and wet or dull conditions (Hubbard, 1954) have been observed to have this effect. Under favourable circumstances, the proportion of florets which set seed seldom exceeds 70% and may often be much less. For example Johnston (1960) showed that as many as 10% of the florets in cocksfoot may be morphologically sterile and thus incapable of developing a seed. However, Knowles and Baenziger (1962) working with bromegrass suggest that infertility is genetic in origin and that the environment also influences the proportion of florets which set seed. Similarly Marshall & Ludlam's (1989) work on *L. perenne* commented that the degree of abortion may be genetic rather than occurring as the result of shortage of nutritional factors.

2.4. Pathogens and disease association

2.4.1. Fungal pathogens of grass crops:

Plant diseases generally affect herbage seed crops more than grazed pasture, as grazing removes much of the diseased plant. In grass seed crops diseased material is not removed and therefore the disease is allowed to incubate (Latch, 1980, Labruyere 1980). There are several leaf diseases which are considered to have a detrimental effect on seed yield of cocksfoot. Stripe rust (*Puccinia striiformis* var. *dactylis* Westend.) is only found in cocksfoot and can seriously affect seed yield. Rolston *et al* (1989) found that fungicide application reduced stem rust in cocksfoot and produced a 600 kg/ha seed yield increase. White (1990) stated that stripe rust can also reduce seed yield. Leaf

scalled (*Rhynchosporium orthosporum* Caldwell) affects both ryegrass and cocksfoot, but is only considered to be moderately severe on cocksfoot. Latch (1980) suggested that rust infection on the stems interferes with the translocation of nutrients to the developing seed, resulting in shrivelled and sometimes non viable seed. Another leaf disease which Ganeb and Harvey (1994) describe as the most common leaf spotting disease in cocksfoot is eyespot (*Mastigosporium rubricosum*). This disease is found on leaves all year round. This is not confused with Drechslera leaf spot (*Drechslera* spp) of which there are six species present on cocksfoot in New Zealand, although all are of little significance. The first symptoms of eye spot are small elliptical water soaked spots about 1 mm in diameter on both sides of the leaf. These become dark brown and are usually surrounded by a yellow to bright orange-tan halo. Lesions enlarge up to 12 mm. Leaves become chlorotic and senesce prematurely. This disease can cause a reduction in green leaf area and seed yield through reduced seed numbers and lowered thousand seed weight.

2.4.2. Bacterial pathogens of grass crops:

The direct pathogenic effect of bacteria on seeds may be referred to under the following four types of disease conditions: seed abortion, seed rot, seed discolouration and 'slime disease'. Depending on the pathogen involved and the weather conditions during seed maturation one or other of these disease conditions may be prevalent in seed or may occur in combination. Several bacteria infect grasses, but few seem to be of great economic importance (Plumb, 1988). Bacterial diseases especially wilt (*Pseudomonas* spp) of forage grasses was first described only 20 years ago (Egli, Goto and Schmidt, 1975). Since then it has been recorded in cocksfoot in the United Kingdom (Wilkins and Exile, 1977); Norway (Sletten, personal communication) and Belgium (Mooter et al, 1981). Outside Europe, New Zealand is the only country where bacterial wilt of forage grasses has been identified (Latch, Young, personal communications). In Scotland, infection is widespread in ryegrass, although its effect on crop yield is slight, but cocksfoot and *Poa. paratensis* have been recorded as being occasionally infected (Channon and Hissett, 1984). In Japan a chocolate spot disease of *L. multiflorum* caused by *Pseudomonas coronafaciens* var *atropurpurea* has been reported (Sakai et al. , 1979). These bacterial pathogens have been reported only rarely and appear to be only sporadic

in appearance. The most important bacterial pathogen *Clavibacter rathayi* has been recorded in cocksfoot from various parts of the world (Smith, 1914; Hardison, 1945; Williams and Taylor, 1957; Skou, 1965; Schlosser, 1969) including New Zealand (Johnston, 1956) and causes Rathay's or yellow slime disease. The main damage is caused by the profuse development of yellow bacterial slime on the inflorescence of plants, often causing dwarfing and distortion. Inflorescence may also fail to emerge from the leaf sheath which are firmly stuck together by slime. It is difficult to estimate the actual loss caused by this pathogen, but in Denmark about 10-50% of cocksfoot seed lots have been found to be infected with *Clavibacter rathayi* (Skou, 1965).

2.4.3. Nematode pathogens of grass crop

The role of nematodes in New Zealand is poorly understood. Two species namely, *Heterodera trifolii* and *Ditylenchus dipsaci* which are both widely distributed throughout the world are known in New Zealand to commonly attack introduced leguminous plants (Grandison, 1963 and Atkinson, 1965). Yeates *et al.*, (1983b) however has listed some plant and/or bacteria feeding nematodes as well as some plant pathogenic nematodes as being important in cocksfoot crops. The cereal cyst nematode (*Heterodera avenae*) can affect ryegrass and cocksfoot when these crops are sown after oats, and *Heterodera* spp. has been shown to be implicated in damage to reseeded grassland (Cook and York, 1980). Increases in herbage yield as a result of decrease in plant feeding nematodes, plant pathogenic nematodes and bacteria feeding nematodes have been reported following the application of nematicides (oxamyl and carbofuran) by Yeates *et al* 1983a; Smolik, 1977 and Stanton *et al* (1981). Usual features of nematode parasitism of ryegrass in Australia is the occurrence of annual ryegrass toxicity. In this condition seeds are replaced by bottle-shaped galls containing nematode larvae. This restricts regeneration of the sward from self-set seeds, but the principle problem is caused by the association of the galls with a bacterium which covers the inflorescence with a sticky, yellow bright slime as reported in New Zealand (Johnston, 1956) and Denmark (Skou, 1965). This makes the seed toxic to sheep and cattle, many of which die after eating infested seed (Price, *et al.*, 1979a) The nematode responsible is *Anguina funesta* (Price, *et al.*, 1979b; Stynes and Bird, 1980), and its effects occurs as a vector of the bacterium *Corynebacterium rathayi* which produces the toxin.

2.4.4 Bacteria and nematode association:

Like the yellow slime disease (or in India tundu disease of wheat) involving the nematode *Anguina tritici* and the bacterium *Clavibacter tritici*, a similar yellow slime or Rathay's disease of cocksfoot, is prevalent in seed crops of cocksfoot in Denmark (Skou, 1965), New Zealand (Johnston, 1956) and the USA. It causes rotting of seeds and produces excessive amounts of bacterial slime which, on drying, become a varnish-like cover of the seed (Sabet, 1954; Vasudeva, 1957). The epidemiology is not fully known since most workers have reported no success after artificial inoculation with either pure culture or slime. The nematode *Anguina tritici*, which transmits the closely related *C. tritici* on wheat, has however, been shown by Sabet (1954) also to carry *C. rathayi* into wheat and cause identical disease symptoms, but this nematode has not been shown to similarly affect cocksfoot. Sabet therefore, suggested that an unknown nematode vector may be involved in transmitting *C. rathayi*. The absence of the necessary vector may explain the frequent failure of inoculation. Direct plant to plant transmission seems unlikely. However, Skou (1965) did establish a clear correlation between the yearly percentage of infected seed and rainfall in the early part of the summer.

2.5. Varieties/ cultivars

The reproductive coefficient of cocksfoot and other grasses varies considerably. For example Martyniak and Domanski (1983) reported that the reproductive coefficient varies between cvs and is highest in cocksfoot and lowest in perennial ryegrass. Seed yield is also closely correlated with precipitation and temperature and varies by >50% with local and seasonal conditions. Also, seed yield stability is often decreased with stand age (Martyniak and Domanski 1983). Plant breeders have accomplished miracles in the development of more useful varieties of grasses. These accomplishments however are of little value unless ultimate seed users have adequate supplies of seed of such varieties (Cowan 1970). Cocksfoot has many fine characteristics which are recognised, but it also has some limitations. It is often too stemmy to provide the best quality forage. Although there are strains which are leafy and provide excellent pasture herbage, most leafy pasture types are poor seed producers and generally lack winter hardiness as described by Wheeler and Hill (1957). Because of the sensitivity of cocksfoot to day length and day-night temperature relations, seed production of new varieties of cocksfoot

is less likely to be successful in latitudes removed from areas of primary adaptation (Wheeler and Hill 1957). Variation in flowering requirements is common in different varieties/strains of cocksfoot as indicated by Calder (1964b).

In 1980 two new cocksfoot cultivars were released by DSIR Grasslands, Palmerston North, to succeed 'Grasslands Apanui'. 'Grassland Kara' is a taller more erect, and more sparsely-tillered plant than cv Apanui. The mature leaves of cv Kara are generally longer, broader, and more bluish than those of cv Apanui. In a sward, cv Kara has wider, longer, erect leaves, and slightly lower shoot density than cv Apanui (Rumball 1982a). Cultivar Kara heads about the same time as cv Apanui, but peak flowering is a few days later (mid- December). There are slightly fewer seed heads than in cv Apanui, and seed yield may be similar or slightly lower with a similar thousand seed weight (1.0 g/1000 seed).

Cultivar Wana is distinctly different in morphology from that of its predecessor. The individual plants are low-crowned, with shorter leaves than that of cv Apanui. The leaves of cv Wana are also lighter and shinier green and foliage is much more resistant to stem rust and stripe rust. In swards cv Wana gives a dense even cover unlike cv Apanui. Cultivar Wana inflorescence are shorter and begin to emerge a few days later than those of cv Apanui, but reach peak flowering at about the same time (mid-December). There are usually more heads per plant in cv Wana, so that although individual seed weights are slightly smaller, the total seed yield may be greater than that of cv Apanui (Rumbel, 1982b).

Compared with cv Kara, cv Wana is more prostrate and densely-tillered, light green and rust free. It flowers a few days earlier and yields more seed. Cultivar Wana is intended for use in sheep pastures, providing summer forage especially where soil fertility and moisture are low (Rumball 1982 b). Cultivar Kara, on the other hand, is intended for use in dairy pastures in seed mixes with other tufted grasses such as timothy, prairie grass, and rye grass, together with clovers (Rumball 1982a).

Cultivar "Grasslands Tekapo" was used here in trial 2 of the present study for an evaluation of the effectiveness of three different types of fungicides. This cultivar is similar in its morphology to cv Wana, being prostrate with similar leaf colour and similar tiller density at heading. The leaf of cv Tekapo is slightly shorter and narrower than that of cv Wana. Due to a slightly shorter and branched panicle than cv Wana, cv Tekapo has slightly lower seed yield potential. Cultivar Tekapo has little winter growth, but vigorous summer growth. Thus it is intended for use in sheep pastures in areas with low fertility and rainfall (Anon 1995).

The remaining two cultivars used in the Trial 1. were cv Makibamidori and Akimidori which come from Japan. No information was available on the morphology or seed producing ability of either of this cultivars. However, Jiro (1989) from Japan reported low wintering ability and large year to year seed yield fluctuation in cv Akimidori.

2.6. Harvesting, threshing and seed cleaning :

The two major problems with harvesting cocksfoot seed are that the seed tends to shake easily once mature and also that there is uneven ripening within the inflorescences. Perhaps the most common method of harvesting cocksfoot is to windrow the crop, allow it to mature in the windrow and then to thresh it with a combine harvester. Cocksfoot is ready to harvest for seed when the stems turn yellow for 15 cm below the head, or shatter when rubbed in the palm of the hand, even though culms and leaves may still be green. Seed shattering is also common in cocksfoot and severe yield losses may occur if the crop is not harvested at the appropriate time. For example Tomov, (1989) from Bulgaria investigating the optimum date of harvest for seeds of cocksfoot cultivar Dbrava indicated that natural seed shattering began at a seed moisture of <43% and reached a daily maximum at 28-31%. Seed gathered at 15-42% SMC (after seasoning in the field) showed similar physical and economic properties. He also showed that up to 47% of potential seed yield could be lost by shattering, but that seed harvest at a moisture content of 35-42% was economically feasible. Pegler, (1976) recorded losses of more than 33% of potential seed yield in S 345 cocksfoot, and Nellist and Rees, (1963) Andersen and Andersen, (1980) reported more than 20% losses due to delayed harvesting. However, Falcinelli *et al*, (1984) reported that cocksfoot populations from

southern and central Italy did not shatter seed even at full maturity and that this character was under genetic control with a high degree of heritability (Falcinelli *et al.* 1989). They concluded that seed retention was not related to moisture content or stem colour and that strong rachillas and densely packed panicles and spikelets appear to be the main cause of this high degree of seed retention. Falcinelli (1987) observed that shattering in cocksfoot is a two-stage process, involving disarticulation of the rachilla, followed by subsequent release of seed from the glumes. The difference between high and low shattering cultivars was found to be in the development of two abscission layers: the primary below the caryopsis and the secondary just above the glumes which was identifiable and quite evident in earlier stages in high shattering cultivars. Cocksfoot seed requires more care, more elaborate equipment and more experienced operators for cleaning than most other grass seeds Wheeler and Hill (1957). It is also extremely difficult to estimate the percentage of clean seed in thresher-run cocksfoot seed so that buyers and sellers are likely to miscalculate the percentage and estimate either much more or much less clean seed than is actually present in a threshed but uncleaned seed lot (Wheeler and Hill 1957).

2.7.1. Use of bactericides

The only effective control of bacterial disease in cocksfoot has been by the use of resistant cultivars. However commercial bactericides have been tested in some forage species with varying results. For example in marram grass (*Ammophila arenaria*) different fungicides (Captan) and nematicides (oxamyl) have all been shown to improve plant growth (Putten *et al.*, 1990). However bactericide (streptomycin and penicillin) application had no effect (Putten *et al.*, 1990). Streptomycin is an antibiotic chemical (streptomycin sulphate), available in wettable powder form, and generally used to control bacterial diseases, and recommended as a foliar spray for fire blight control in New Zealand at 60g/100l at 7-10 day interval (Anon. 1993). Ingham *et al.*, (1991) have also observed the effect of applications of streptomycin and also captan in no tillage agroecosystems which have indicated that treatment of soil with streptomycin results in a reduction in the bacterial population but also in an increase plant death in longer term grass swards within 2-4 weeks. Phytotoxicity due to the application of streptomycin has also been reported indicating some mild toxicity on some plants and the need to restrict the use of streptomycin as a foliar spray to concentrations below 400ppm (Anon. 1976)

2.7.2. Use of nematicides

Nematodes are always present in grass crops. Both parasitic and freeliving nematodes are reported to cause damage either alone or in combination with other pathogens in the soil as well as on above ground parts (Bezooijen 1979). Nematicides such as oxamyl (Vydate), carbofuran, aldicarb (Temik) have all been reported to increase crop growth as well as crop establishment by reducing nematode populations (Yeates *et al.* 1983a; Bezooijen, 1984; Spaul and Mewton 1984). Vydate, a carbamate insecticide/ nematicide (oxamyl) is available as a water soluble concentrate used to control root knot, lesion and foliar nematodes of ornamental, pasture and pipfruits, in New Zealand when applied at 350ml/100L at 14 day intervals. This pesticide is compatible with most fungicides and insecticides except alkaline materials (Anon. 1993). Bezooijen (1984) stated that application of vydate as a liquid slow release formulation at 2.5 Kg or 5 Kg a.i./ha to grass crops in the Netherlands resulted in yield increases, even though nematode populations were not reduced. It was suggested however that the chemical inactivated nematodes by a repellent action giving the plant a chance to establish. Bezooijen *et al* (1989) also stated that infected ryegrass crops following application of oxamyl at 1 and 2 kg ai/ha, resulted an average herbage yield increases of about 15% in ryegrass infected with *Tylenchorhynchus*, *Paratylenchus*, *Pratylenchus*, *Heterodera*, and *Meloidogyne* spp. Clements *et al* (1985), working with italian ryegrass tested 18 pesticides and stated that among those tested Oxamyl (granules) at 2 or 4 kg a.i./h was most effective and increased herbage yield by > 0.4 t/h. Similarly Spaul and Mewton (1984) investigating losses in yield of grasses and cereals caused by nematodes reported that herbage yields were significantly greater when oxamyl was applied. They suggested that each doubling of nematode numbers reduced grain yield by about 0.8 t/h. Vydate (oxamyl) is also a good insecticide and in sometimes used to prevent or control soil borne insect pests. For example Barker *et al*, (1984) investigating the effect of stem weevil in cocksfoot crops, indicated that both oxamyl and carbofuran treatment increased cocksfoot forage yield by 15-19%

2.8. Use of fungicides in grass seed crops

2.8.1. Causes of seed yield increase by fungicides

The use of fungicides, such as propiconazole, on grass seed crops has increased over the last decade, as growers have become more aware of the seed yield losses associated with

diseases, such as stem rust (*Puccinia graminis*) or eye spot (*Mastigosporium rubricosum*). Fungicide application has been found to increase seed yield in perennial ryegrass (Hampton and Hebblethwaite, 1984; Hampton, 1986; Horeman, 1989; Welty, 1989b, 1990), cocksfoot (Rolston, *et al* 1989; Welty, 1989a) and prairie grass (Rolston *et al*, 1989).

Even when disease incidence has been extremely low or even absent, fungicide application has still been shown to increase seed yields in perennial ryegrass (Hampton and Hebblethwaite, 1984; 1985; Hampton *et al* 1985) and cocksfoot (Rolston *et al.*, 1989).

Many heavy yielding grass seed crops lodge soon after ear emergence or at or about anthesis. Lodging creates a microclimate which can promote fungal growth (Griffiths, 1969) and encourage stem and leaf diseases such as *Drechslera* sp., *Erysiphe graminis*, *Puccinia coronata* and *P. graminis*. If such pathogens are present, they may destroy fertile tillers, reduce photosynthetic capability, disrupt assimilate supply, cause complete leaf decay, destroy seed and seed heads, and lower seed weight (Griffiths, 1969).

It is perhaps surprising that where there has been little or no disease out break, fungicides have still increased seed yields. These yield responses have apparently been due to increased green leaf area duration during anthesis and seed development, brought about by fungicides delaying the senescence of photosynthetic tissue as described by Hampton and Hebblethwaite, (1984) and Rolston *et al.*, (1989).

The period between anthesis and seed harvesting in grass is particularly influenced by early application of fungicide which creates a direct response by increasing numbers of seeds per spikelet and seed weight (Hampton and Hebblethwaite, 1984, 1985). Other seed yield components generally have not been affected, although most researchers have not reported the number of spikelets per tiller or seeds per spikelets after anthesis and at seed harvest. Hampton and Hebblethwaite, (1984, 1985) and Hampton (1986) are the only reports in which seed component data just before or at harvest have been presented. Such data at harvest are of extreme importance since they enable clear explanations of

the positive effects of fungicides in reducing the high levels of seed abortion of developing seeds which occurs in the absence of fungicide (Hampton and Hebblethwaite, 1984).

2.8.2. Types of fungicide and rates

The most widely used and effective fungicide in recent work has been propiconazole (Horeman, 1989; Rolston *et al.*, 1989; Welty 1989 a,b,c, 1990) This is the only recommended fungicides for use in ryegrass seed crops in New Zealand, an application rate of 125 g ai ha¹ being recommended for the control of stem and leaf rust (O'Connor, 1989). In Oregon, however, propiconazole, chlorothalonil and triadimefon are all registered for use in controlling foliar diseases of grasses grown for seed (Welty, 1989b). New triazoles (terbuconazole, cypronazole and hexaconazole) have all been reported to be more efficient than propiconazole, because of greater persistency (Clinkspoor, 1991). One of these new fungicides is Folicur (terbuconazole) a triazole available as an emulsifiable concentrate. This chemical is generally used in New Zealand to control stripe rust, leaf rust, speckled leaf blotch (wheat), scald, net blotch, spot blotch (barley); crown rust, and stem rust in ryegrass at 750ml/ha at 21 day intervals Anon. (1993). Alto 100 SL (cyproconazole) another triazole, is also available as a soluble concentrate, and is reported in New Zealand to control stripe rust, leaf rust, speckled leaf blotch and blossom blight in wheat and ryegrass, when applied at 400ml/ha at 21 day intervals (Anon. 1993). Bavistin WP (carbendazim) benzimidazole is also available as a wettable powder, and is recommended in New Zealand for the control of sclerotinia in lettuce when applied as a foliar spray. This chemical also controls eyespot in wheat and barley, and scald in barley when applied at 2.2 kg/800L /ha at 14 day intervals (Anon. 1993). Hampton and Hebblethwaite (1984) have also used carbendazim in ryegrass seed crops and reported increases in yield. Bravo (chloroethalonil) a nitrile group chemical is available as a suspended concentrate and is recommended in New Zealand to control rust in beans, leaf spot in blackcurrants, septoria leaf spot in celery and leaf spot disease in ornamental crops, when applied at 3 l/ha at 14 day intervals (Anon., 1993). Welty (1989b) has also shown that one or more applications of chloroethalonil results in significantly higher seed yields in cocksfoot.

2.8.3. Timing of fungicide application

Hampton and Hebblethwaite (1984) applied fungicide (triadimefon plus carbendazim plus captafol) at 100 g m⁻² in two applications (at ear emergence and just before anthesis) and five to six times at monthly intervals from spring tillering until seed harvest, to a perennial ryegrass seed crop. They recorded a significant increase in seed yield. In later work, Hampton (1986) showed significant seed yield responses from these fungicides applied either once (at ear emergence) or twice (at ear emergence and just before anthesis) in perennial ryegrass.

Rolston *et al.*(1989) obtained significant seed yield increases in prairie grass with propiconazole applied once (after ear emergence), or twice (after ear emergence and immediately after peak anthesis) but not when applied only after anthesis. They also obtained increased seed yield in cocksfoot using propiconazole or mancozeb applied once at early anthesis.

In cocksfoot, Welty (1989a) found that one or more applications of chlorothalonil at the "boot" or heading stage (ear emergence) resulted in significantly higher seed yields compared with untreated plants. Welty (1990) also found a significant increase in one cultivar of perennial ryegrass but not in another when propiconazole was applied twice during ear emergence. Horeman (1989) found that applying fenpropimorph or propiconazole three times (early ear emergence, before and after anthesis) was more effective in increasing seed yield than fewer applications. However, generally fungicide application at ear emergence, before the onset of disease, appears to be most effective in controlling disease and preventing seed yield losses. Fungicide applied after anthesis is generally least effective.

2.9. Seed quality

Seed quality refers to a collection of seed properties which are considered to be important for the value of seed for sowing purposes (Esbo, 1980). Thompson (1979) outlined 10 seed quality components with varying degrees of practical importance to agriculture viz. analytical purity, species purity, freedom from weeds, cultivar purity, germination capacity, vigour, size, uniformity, health and moisture content. Scott and

Hampton (1985), based on trials in cocksfoot have suggested that seed weight should be introduced particularly for cocksfoot as a criteria for quality. For example, Charlton and Thom (1984) observed that the establishment of the cocksfoot cultivar Wana could be improved if the number of multiple seed units in the seedlot was reduced. Scott and Hampton (1984) also indicated that seedlot germination could be increased by reducing the percentage of multiple seed units and increasing mean seed weight. Historically, seed quality has been synonymous with germination. The basic aim of germination testing is to provide information about the planting value of the seed lot. Although both season and region of production can strongly influence germination results (Hampton and Young, 1985), infestation by pests and diseases has always been considered to be a limiting factor in reducing both seed yield and quality.

CHAPTER III

MATERIALS AND METHODS

3.1. Site and treatment

The trials were conducted at the AgResearch Grasslands lowlands research station, Aorangi (latitude 40° S) near Palmerston North, New Zealand, on a Holocene siliceous sandy alluvium soil using a cocksfoot seed stand coming up for its third annual harvest. Originally the trial site was cultivated and sown out of a three year old ryegrass/white clover pasture. The five cultivars used were sown by hand on 23rd October, 1991 at a rate equivalent to 3 kg/ha. The unit plot size was 3 X 1.2m and row width was 30 cm. After seed harvest in the second year (23rd December-05th January, 1994) nitrogen was applied twice (at @ 50Kg N/ha on 15th August, 1994 and 100 Kg N/ha on 28th October 1994). The trial design for experiment 1 was a Complete Randomized Block Design (CRBD) incorporating four cultivars (Kara, Wana, Makibamidori and Akimidori) and 4 pesticide treatments (Streptomycin, Vydate, Streptomycin + Vydate and Folicur) (Table 1). The trial design for experiment 2 was also a Complete Randomized Block Design but using only one cultivar (Tekapo) with 4 fungicide treatments. Cultural management and experimental details are presented in Table 1. Weather data at Aorangi (40° S) during the cropping months are presented in Appendices 1, 2 and 3.

3.1.1 Trial 1.

In trial 1 the fungicide Folicur (terbuconazole) was applied as a control to all plots at @ 750ml/ha at 21 day intervals from 19th October 1994 for cv Akimidori (early flowering type) and from 17th November for the remaining 3 cultivars (Kara, Wana and Makibamidori). Folicur was sprayed on all plots using a pressurised knapsack sprayer. Bactericide, (streptomycin) was applied to eight (4 for Streptomycin alone and 4 for combination of Streptomycin + Vydate) randomly selected plots of each cultivar in each block at 60g/100 l at 7 day intervals mixed with a sticker 'raingard' at 32.5 ml/100 l spray mixture. The nematicide Vydate (oxamyl) was applied to 8 plots at 350ml/100 l (4 for Vydate alone and 4 for the combinations of Vydate + Streptomycin) in each cultivar along with raingard. The remaining 4 plots of each cultivar received fungicide Folicur alone which served as the control treatment for the experiment.

3.1.2. Trial 2

In trial 2 three Fungicides Alto (Cyproconazole), Bavistin (Carbendazim), and Bravo (Chlorothalonil) plus a control (tap water) were applied to 3 randomly selected plots in each block at 400ml/ha at 21 day intervals for Alto; at 2.2Kg/800 l/ha at 14 day intervals for Bavistin and at 3l/ha at 14 day intervals for Bravo. All Fungicides and tap water for the control treatment were applied using a pressurised knapsack sprayer and were tested on one cultivar (cv Tekapo).

Fig 1. Experimental design plot-plan



Experiment 1.

Pesticides used Streptomycin = T1, Vydate = T2, Control(Folicur) = T3; Streptomycin+Vydate = T4

Experiment 2.

Fungicides used : Alto = T1, Bavistin = T2, Control = T3, Bravo = T4,

Table 1. Cultural management and experimental details.

Managements		location and Date
Site		AgResearch Aorangi Farm in Manawatu
Soil type		Holocene siliceous sandy alluvium
Sowing rate		3kg/ha
Sowing date		23rd October,1991
Age of crop		3 years (half of the plots were sown on 23rd October, 1991 & the remainder in April 1992)
Plot Size		3 x 1.2 m with 30cm row width
Design		Trial 1. RCBD with 2 factors Trial 2. RCBD with 4 treatments
Replications		Four
Fertilizer		Nitrogen applied twice @ 50Kg N/ha on 15th August, 1994 and 100 Kg N/ha on 28th October, 1994 as urea.
Last grazing or cut		All plots were mown in mid February, 1994
Weed Control		Initially Weeds were removed mechanically on 5.8.94 then sprayed twice with herbicide 'Buster' on 27-10-94 @ 5.4l/ha and on 15.12.94 @ 6.3l/ha.
Insect Control		Nil
Seed Harvest		23rd December - 05th January
Treatments for Expt.1	19.10.94 to 29.12.94 at weekly intervals	Streptomycin at 7 day intervals @ 60g/100l.
	19.10.94 02.11.94 17.11.94 01.12.94 15.12.94 29.12.94	Vydate at 14 day intervals @ 350 ml/100l.
	same as above	Combination : Streptomycin + Vydate at individual rate as above
	19.10.94 17.11.94 08.12.94 29.12.94	Folicur at 21 day intervals @ 750ml/ha
Cultivar used		Wana, Kara, Makibamidori and Akimidori
Spray starts from		19th October for cv Akimidori and 17th November for other 3 cultivars
Treatments for Expt.2	10.11.94 01.12.94 22.12.94	Alto at 21 day intervals @ 400ml/ha
	19.10.94 24.11.94 08.12.94 22.12.94	Bravo at 14 days intervals @ 3l/ha
	Same dates as Bravo	Bavistin 14 day intervals @ 2.2Kg/800l/ha
Cultivar used		Tekapo
Spray starts from		10.11.94
Additive chemical		Raingard @ 32.5 ml/100l spray Mix.
Seed Harvest		5th January, 1995

3. 2. Foliar disease assessment :

A 1-5 modified scoring scale (Plate 1) as presented by Saari Prescott (1975) was used for evaluating foliar disease incidence on ten randomly selected plants in each plot. The assessment of foliar diseases was made at 3 growth stages ie. at full growth stage (before flowering) 17.10.94, at peak flowering (1.12.94) and at seed maturity (23.12.94).

3.3. Nematode isolation :

Four grams of leaf and stem tissue were chopped (approx. 2cm length), and placed in plastic dishes containing water for 72 hours. Nematodes were then separated from the water with a 63 μ m aperture sieve. For general survey nematodes from soil were also collected following the simple incubation technique described by Rodigue-Kabana and Pope (1981)

3.4. Bacteria isolation and inoculation :

Liquid suspensions containing macerated seed heads of cocksfoot (misshapen or discoloured) were grown on different selective media viz. Nutrient Broth Yeast Extract (NBE), Nutrient Glucose Agar (NGA), TTC Diagnostic Media and a Semi Selective medium for the detection of *Clavibacter michiganense* subsp. *michiganensis* (Hassan Bolkan Pers. communication). Plates were incubated at 25⁰C for 48 hours and colony characters compared with an authenticated culture of *C. rathayi* obtained from cocksfoot supplied by the International Collection of Microorganisms from Plants (ICMP), Auckland, New Zealand. Detailed recipes of all media used for growing bacteria are given in Appendix 17. Inoculum suspensions from freshly incubated colonies of both the authenticated culture and isolated bacteria were sprayed alone and in combination with isolated nematodes on to healthy cocksfoot plants grown in pots in a glasshouse to attempt to duplicate seedhead disease symptoms.

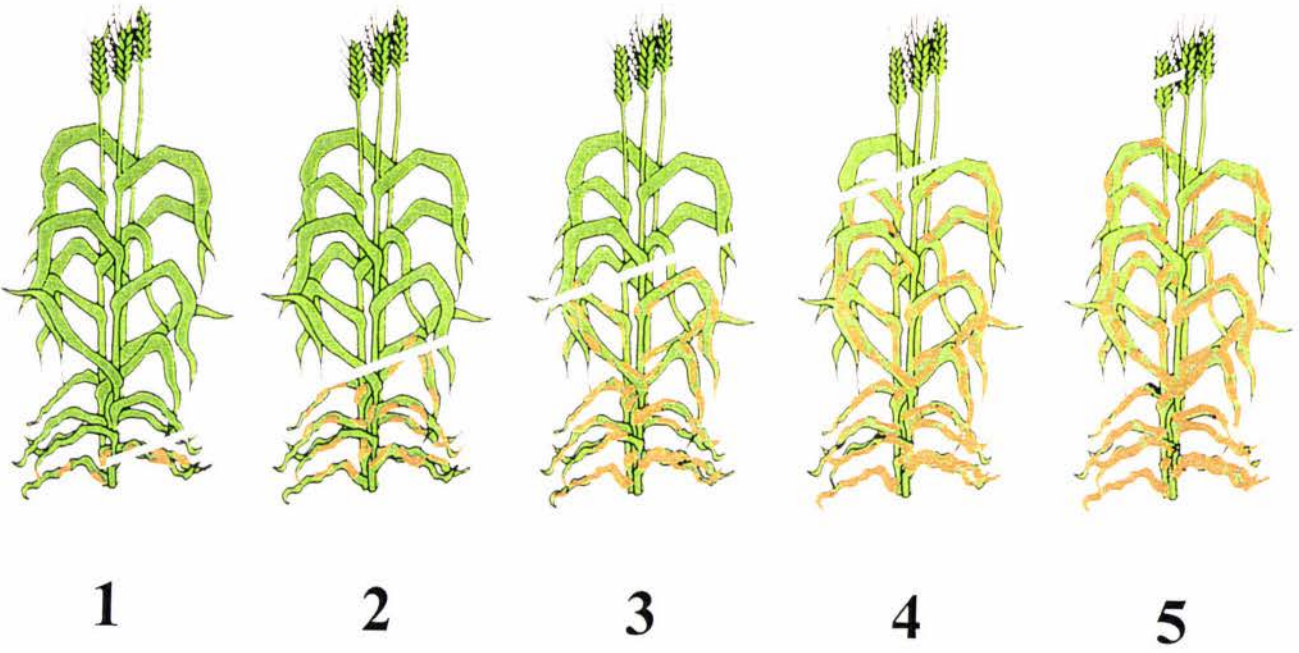


Plate 1. Disease scoring scale (from Saari Prescott 1975)

3.5. Seed yield, seed yield components and harvest

Data on number of fertile tillers/m² was obtained by placing a 0.1 m² steel frame on individual plots and counting all fertile tillers. Five reproductive tillers were randomly cut from each plot at peak anthesis (1.12.94) for spikelet and floret counts. All spikelets on each of the 5 heads were counted with the average of five heads being recorded. The average number of florets per spikelet was determined by counting the number of florets in randomly selected individual spikelets from the top, middle and bottom of the head. The average multiplied by the number of spikelets gave the number of florets/head. Pure seed yield was determined from the percentage by weight of pure seed present in a 1.5g working sample. Pure Seed yield/m² were determined by adding the total weight of pure seed present in each cleaned fraction from the plot sample and adjusting the total to a constant seed moisture content of 10%.

3.6. Seed harvesting and cleaning

Seed was harvested from 23rd December - 05th January, 1995 (depending on cultivar) by hand, by removal of all heads from the entire plot with a hand shear when seed moisture was about 40%. The cut heads from each plot were placed in individual hessian sacks, and the sacks labelled. Seed heads from each individual sacks were then placed on the top of flat tables at ambient room temperature and air dried for three weeks to a seed moisture content of approximately 14% before being threshed using a small belt thresher, which consisted of two rubber belts, one travelling on the top of the other in the same direction but at different speed which produced a rubbing action and removes the seed from the seedhead (Plate 2) at Ag Research Palmerston North, New Zealand. Seed lots were initially sieved on a Clipper (USA) two screen air screen cleaner to remove unwanted leaf and stem material. Care was taken to ensure that each sample remained completely separate, with the cleaner being carefully cleaned and vacuumed between samples. The top screen had 5.5mm x 20mm elongated holes, and the bottom screen 2mm x 5.5mm elongated holes. After the removal of unwanted trash the seed was further cleaned with a 5.50 mm. indented cylinder (Westrup, Denmark) at the Seed Technology Centre, Massey University. The left over fractions from the air screen cleaner together with that from the indented cylinder were kept in separate packets (Screen fraction) and labelled to determine the pure seed losses in commercial cleaning. Finally light chaffy material was removed using a Contab vertical column blower and the leftover fraction from the blower (Blow fraction) was also kept in another packet for determination of cleaning losses.

After cleaning the seed, each sample was weighed on a Mettler (PE 3600) balance and data were recorded for each plot. After the yield had been recorded the seed moisture content was determined using the constant temperature oven method (ISTA 1993). For each sample, two empty containers and lids were weighed (m1), then reweighed with the seed in it (m2). The container was then placed in an oven at a constant temperature of 130°C for 1 hour. All containers were removed, placed in a desiccator for 10-15 minutes to cool before being reweighed (m3). The seed moisture content was calculated as follows:

$$(m2-m3) \times 100 / (m2-m1)$$

From the cleaned seed and separated fractions a minimum of 1.5 g working sample was prepared using a Precision Divider (Dean Gamet, USA) each had been estimated to contain a minimum of 2500 seeds which fulfils seed testing requirements (ISTA 1993). From this working samples the analytical purity and germination (Single and multiple floret) of each lot was determined using international standardised methodology (ISTA, 1993). When these working samples were not in use they were stored in polythene bags at 5°C.



Plate 2. Rubber belt thresher used for threshing

3.7. Seed quality:

3.7.1 Germination Test:

A test sample of 4X50 pure seeds from both single and multiple florets obtained from the purity test working sample was used for the germination test. The top of paper (TP) method was used. Each lot was placed on top of moist paper (90mm x 90mm) which was then placed in a plastic container 125 mm x 225 mm x 2 mm. In each container two lots of 50 seeds were germinated. A lid was then firmly placed on top of the container to prevent moisture loss prior to prechilling at 5^o C for 4 days for cv Tekapo, Makibamidori and Akimidori or 7 days for cv Kara and Wana as recommended by MAF Seed Testing Station, Palmerston North, New Zealand. After prechilling, the containers were placed in a germination cabinet (Warren Sherer KY50R controlled environment germinator) at alternating temperature of 20^o C for 16 hour without light and 8 hours at 30^oC with light for a total period of 21 days (ISTA 1993). An initial count was made after 10 days and a final count at 21 days following the ISTA Rules (ISTA, 1993). The classification of normal and abnormal seedlings and fresh ungerminated seeds was used as described by ISTA (1993)

3.7.2 Seed weight:

The pure seed was used to determine thousand seed weight. Four replicates of 100 seeds were weighed separately from each sample using a Sartorius balance (3 decimal places). The average 100 seed weight between replicates was used to determine 1000 seed weight.

3.7.3. Purity

To calculate the average purity of a sample, each working sample was placed in a Micro-blower type 35 (Plate 3) and exposed to a 11.5m/sec constant air blast for two minutes. The pure single floret (seed), multiple floret (seed) weed seed and inert matter were then weighed to two decimal places using a Mettler PE 3600 balance. The multiple seed units present were separated by hand and percentage present was determined. By dividing the pure sample weight by the total weight of the working sample and multiplying by 100 the percentage pure seed in each working sample was calculated.

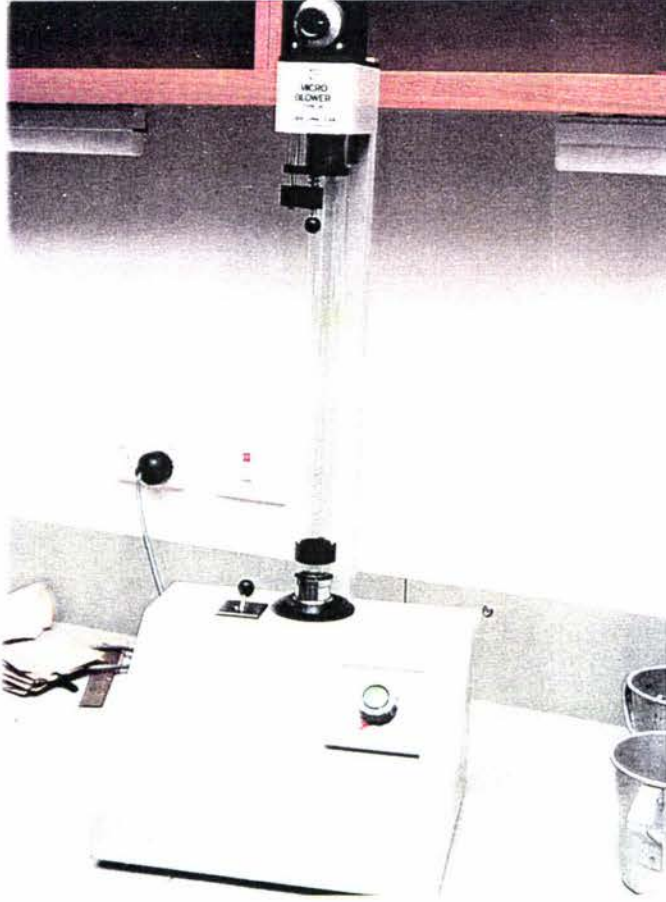


Plate 3. Micro blower type 35

3.8. Statistical analysis and graph :

Statistical analysis was carried out using the SAS system (SAS, 1990). Least Significant Difference with probability 5 percent ($LSD_{0.05}$) was used to differentiate data where analysis of variance (ANOVA) or general linear model (GLM) was significant at 0.05, 0.01 or 0.001 levels. Graphs were made using the GLE Soft Ware Programme.

CHAPTER IV

RESULTS

Trial No.1

4.1. Effects of cultivar

Analysis of variance showed significant differences in seed yield and seed yield components between cultivars (Table 2 and 3). Differences were also found during seed cleaning and significant variations observed between pure seed levels recovered from different fractions after cleaning (Table 5). Foliar disease levels and nematode populations in the field did not greatly change in each cultivar although some significant differences in seed quality were obtained (Tables 6 and 7).

4.1.1. Seed yield and yield components

Seed yield

The mature threshed harvested seed yield/m² of each cultivar is presented in Table 2. Cultivar Wana (113.5 g/m²) significantly outyielded the other three cultivars, being 51.3% higher than in cv Akimidori. Seed yield in cvs Kara and Makibamidori was similar but were both significantly lower than cv Wana and significantly higher than cv Akimidori. The data obtained from this experiment (Table 2) suggests the considerable variation in seed yield capacity between the cultivars with the two New Zealand cultivars outyielding (Wana), or similar (Kara), to Makibamidori but significantly outyielding the Japanese cultivar Akimidori. Seed yield in cv Akimidori was particularly poor, probably suggesting uneconomic seed returns from this cultivar under New Zealand conditions.

Table 2. Differences in mean harvested yield between cultivars.

Variety	Harvested Seed yield g/m ²	Pure Seed yield g/m ²	% of Pure Seed Recovered
Wana	113.50 A	79.95 A	69.58 AB
Kara	82.67 B	61.19 B	72.52 A
Makibamidori	79.78 B	53.87 B	66.93 B
Akimidori	55.27 C	23.90 C	43.18 C
LSD p<0.05	18.49	12.92	4.40

** Means are average of 16 observations

The pure seed yield/m² (Machine cleaned seed + pure seed recovered from different fractions) from different cultivars and the % recovered as a % of total harvested seed are presented in Table 2. Mean pure seed yield per m² follows the same order as total harvested yield/m². (Table 2). Cultivar Wana again produced the highest pure seed yield being 70% higher than the lowest yielder cv Akimidori (Table 2). It is interesting to note here that although cv Wana yielded 51.3% higher harvested yield, pure seed yield is 70% higher than cv Akimidori. Similarly the pure seed recovered from harvested material in cv Akimidori is also lowest (Table 2) indicating more empty florets or more abortion in cv Akimidori than in Wana. A similar but less obvious comparison can be made between cvs Kara and Makibamidori. The data obtained from this experiment suggests that seed production in cocksfoot varies depending on cultivar and in this instance cv Wana yielded 70%, 32% and 24% more pure seed per unit area than cvs Akimidori, Makibamidori and Kara respectively. The % of pure seed recovered from the harvested material is also observed to be significantly higher in cvs Kara than cvs Makibamidori and Makibamidori compared to Akimidori (Table 2).

Yield components

Among the different seed yield components number of fertile tiller/m², number of spikelets/head, number of florets/spikelets, total number of florets/head and thousand seed weight (TSW) were recorded. Most of these yield components were found to be vary significantly between cultivars.

Cultivars varied in their fertile tiller production (Table 3). Cultivar Wana produced significantly (approximately 20%) more fertile tillers/m² than the other cultivars. No difference in fertile tiller numbers occurred between cvs Kara, Makibamidori and Akimidori. It is interesting to note here that although the average number of fertile tillers in cv Kara was the same as in cv Akimidori the former yielded significantly higher amounts of seed (Table 2). This higher yield in cv Kara came from significantly higher seed weight as well as a higher number of florets/head.

Table 3. Effect of cultivar on seed yield components.

Cultivar	Fertile tiller/ m²	No. Spikelets/ head	No. of florets/spikelets	No florets/ head	TSW g
Wana	682.0 A	410.56 A	4.51 B	1845.29 A	0.095 C
Kara	555.5 B	358.46 B	4.71 A	1681.43 AB	0.112 A
Makibamidori	566.4 B	324.81 BC	4.58 AB	1488.90 BC	0.111 A
Akimidori	563.8 B	306.33 C	4.62 AB	1413.16 C	0.105 B
LSD p<0.05	85.35	43.06	0.18	193.31	0.004

**** Means are average of 16 observations**

The data presented in Table 3. indicates that mean number of spikelets per head was significantly higher in cv Wana than in the other three cultivars. The difference in mean spikelet number between cvs Kara and Makibamidori and between cvs Makibamidori and Akimidori were not significant however, cv Kara produced significantly higher numbers of spikelets than cv Akimidori. Over 25% more spikelets per head was obtained in cv Wana (410.56) than in cv Akimidori (306.33). The data suggests that although seed weight was higher in cv Akimidori than in Wana, the latter achieved higher yields as a result of greater head numbers, and more spikelets and florets/head. Despite the heavier seed produced by cv Kara and Makibamidori this was insufficient to counteract the lower number of spikelets and seed heads in these two cultivars compared with cv Wana.

The average number of florets produced per spikelet (Table 3) varied from 4.51 (Wana) to 4.71 (Kara) suggesting this yield component is relatively insensitive to change between cultivars and is unlikely to be a component which greatly influences seed yield. The data observed here suggests that the higher yield observed in cv Wana came mainly from increased numbers of seed heads and spikelets, and from higher numbers of florets/head rather than from increase in florets per spikelets or increased seed weight.

The total number of florets produced per head is one of the most important components of seed yield in cocksfoot and was observed to vary significantly depending on cultivar

(Table 3). Cultivars Wana and Kara produced more florets compared to the two Japanese cultivars (Makibamidori and Akimidori). Cultivar Wana produced almost 25% more florets while cv Kara produced 20% more florets per head than in cv Akimidori. This clearly suggests that the lower yield observed in the two Japanese cultivar is associated with lower numbers of florets per head, mainly due to the production of fewer spikelets.

Differences in mean TSW between cultivars are presented in Table 3. Although cv Wana was the highest seed yielder its TSW was significantly lower than in the other 3 cultivars. No differences in TSW were obtained between cv Kara and Makibamidori but both were significantly higher than in cvs Akimidori and Wana. This indicates that cocksfoot cultivars vary in their seed weight (TSW), and that higher yield in cv Wana came from more fertile tillers, spikelets/head and florets present per seed head.

Relationship between seed yield and different Yield components in four cocksfoot cultivars.

Linear correlation coefficients were calculated to determine if yield components were related to seed yield (Table 4). Highly significant and positive coefficients were obtained with fertile tiller number in cvs Kara, Wana and Makibamidori. Although a positive correlation also exists with cv Akimidori the relationship was not significant (Table 4). Thousand seed weight and florets/spikelet were found to be negatively correlated with seed yield. It was therefore the number of reproductive tillers at anthesis, not florets/spikelet or TSW that had the most significant influence in determining seed yield in cocksfoot; the higher the reproductive tiller number the higher the seed yield (Table 3) especially in cvs Kara, Wana and Makibamidori. The value of the coefficient of correlation was high in cv Wana followed by Makibamidori and Kara respectively (Table 4).

Table 4. Simple correlation coefficient for pure seed yield against yield components in four cultivars.

	Wana	Kara	Makibamidori	Akimidori
TSW	-0.691	-0.267	-0.462	-0.049
Floret/spikelet	-0.442	-0.144	-0.090	-0.074
Spikelet/head	0.423	0.624*	0.354	0.190
Florets/head	0.273	0.477	0.454	0.144
Fertile tiller	0.916**	0.728**	0.841**	0.340

Significance at 15 df.

4.1.2. Seed cleaning and seed recovery

Cocksfoot produces "chaffy" seeds which may make it difficult to machine clean and a considerable amount of pure seed is often lost during commercial seed cleaning. Clean seed obtained from cleaning (commercial) procedures (clean seed) and the leftover fraction from each step of cleaning ie. from screen cleaner (screen fraction) and from blower (blow fraction) were kept separate in order to determine the loss of pure seed during the commercial cleaning process by comparison with results obtained using a calibrated sample at a constant air flow.

Observations with the four cocksfoot cultivars showed that a considerable amount of pure seed is lost in the seed cleaning process (both from commercially cleaned seed, screen fraction and air blow fraction). Pure seed yield obtained from these 3 separations are presented in Table 5. Data indicates that significantly higher amounts of pure seed yield were obtained from cv Makibamidori than Wana and Akimidori, suggesting that although the total pure seed yield/m² was higher in cv Wana this cultivar loses higher amounts of pure seed in the machine cleaning process. However, the pure seed yield recorded in commercially clean seed lots of cvs Kara and Wana were similar. This result also shows that the recovery of pure seed in machine cleaning of cv Makibamidori is more than double to that of cv Akimidori and 20-25% higher than cvs Kara and Wana. The total weight of clean sample was also high in cv Makibamidori and about 47% higher than cv Akimidori.

Table 5. Effect of cultivar on pure seed yield (g/m²) from different fractions in commercial cleaning.

Variety	Total clean sample g/m ²	Pure Seed yield g/m ² (clean fraction) ¹	Total screen sample g/m ²	Pure Seed yield g/m ² (Screen fraction) ¹	Total blow sample g/m ²	Pure Seed yield g/m ² (Blow fraction) ¹
Wana	31.79 AB	23.44 B (74.04)	23.43 A	15.86 A (63.54)	58.28 A	40.65 A (68.60)
Kara	31.20 AB	24.93 AB (75.27)	20.25 A	14.99 A (71.91)	31.21 B	21.28 B (62.25)
Makibamidori	42.19 A	31.46 A (74.69)	19.56 A	14.42 A (70.48)	18.03 B	7.99 C (38.58)
Akimidori	22.46 B	12.53 C (58.28)	12.42 B	5.87 B (47.12)	20.39 B	4.93 C (19.87)
LSD p<0.05	10.66	6.67	6.41	4.95	14.10	9.29

¹ Figure in parenthesis indicates % purity

** Means are average of 16 observations

The pure seed yield recovered from the screen fraction material also varied significantly between cultivars. Significantly higher amounts of pure seed were obtained in cvs Wana, Kara, and Makibamidori than in Akimidori by almost 60%. This data on the pure seed yield from the screen fraction once again clearly indicates the higher chance of pure seed loss during machine cleaning. The total weight of screen sample also follows the same pattern and is similar in cvs Wana, Kara and Makibamidori, but significantly higher than in cv Akimidori.

The pure seed yield obtained from the blow fraction as presented in Table 5 also indicates considerable variation between the 4 cocksfoot cultivars. In this instance higher pure seed recovery was obtained in cv Wana than that in cvs Kara, Makibamidori and Akimidori. However no differences in yield were observed in the two Japanese cultivars (Table 5). This again strongly suggests that the cultivar Wana is very much more susceptible to losses of pure seed in the seed cleaning process (screen and blower) than the other cultivars. In contrast cv Makibamidori was less susceptible to cleaning losses in terms of pure seed even though this cultivar actually produced significantly less harvested seed than cv Wana. The total weight of the blow sample was significantly higher in cv Wana than the other three cultivars.

4.1.3. Disease and nematode population

The incidence of foliar disease (eye spot) in each cocksfoot cultivar was assessed visually at three times during the experimental period (17.11.94; 1.12.94 and 23.12.94). The data on overall disease incidence is presented in Appendix 4. (at vegetative growth stage, at peak anthesis and at seed harvest maturity). Overall, eye spot disease incidence in all cultivars was very low. Similarly the presence of freeliving, fungal/bacteria feeding nematodes (suspected vector) on green leaf or stem tissue were estimated before treating with pesticide and at the time of harvest and was found to be similar (Appendix 4). This showed that the population of nematodes was similar in all cultivars ie. these nematodes were distributed uniformly in the field irrespective of cultivar.

4.1.4. Seed quality

Different components of seed quality were affected significantly due to difference in cultivars. Although no major differences in the purity of machine cleaning occurred in cvs Kara, Makibamidori and Wana all were significantly higher in purity than cv Akimidori. The lower pure seed yield in this latter cultivar (58.3%) was further evidence that floret sterility or abortion at different seed development stages resulted in lower % pure seed levels in Akimidori being around 20 % less than in the other three cultivars (Table 6).

Table 6. Effect of 4 cocksfoot cultivar on % pure seed during commercial cleaning.

Variety	% Pure Seed (Air Screen Cleaned Sample)	% Pure Seed (Screened Sample)	% Pure Seed (Blow Sample)	% Multiple flore
Wana	74.04 A	63.54 B	68.60 A	23.98 AB
Kara	75.27 A	71.91 A	62.25 A	17.06 C
Makibamidori	74.69 A	70.48 A	38.58 B	20.78 BC
Akimidori	58.28 B	47.12 C	19.87 C	28.10 A
LSD p<0.05	9.10	4.95	9.86	4.93

** Means are average of 16 observations

The % pure seed present in the screen fraction material (Table 6) showed that cv Kara and Makibamidori and Wana again had a significantly higher % purity than cv Akimidori. This indicates that the % of pure seed present in the screen fraction is almost the same or slightly less (around 70 % in cv Kara and Makibamidori) than the % of pure seed in machine cleaned seed.

Observation on % pure seed present in the blow fraction material showed that significantly higher % of pure seed was present (Table 6) in the two New Zealand cultivars which also ranked top in terms of total harvested seed yield/m² (Table 2). This suggests that the high % of pure seed present in the blow fraction contribute significantly to total seed yield. In the two Japanese cultivars % pure seed present in blow fraction material was low which indicates that the lower seed yield (Pure seed yield/unit area) observed in the two Japanese cultivar was associated with a lower % of pure seed present in the blow left over (Table 6) as well as in screen fraction material for cv Akimidori.

Pure seed in cocksfoot consists of both single and multiple florets (multiple seed units). The quality of seed also depends on the proportion of single and multiple florets which may vary depending on cultivar (Table 6). Certainly cvs Wana and Akimidori had significantly higher proportions of multiple florets than cv Kara but the level is lower in Makibamidori than in Akimidori. However, difference between cvs Akimidori and Wana and between Makibamidori and Kara were not significant. This suggests that production of multiple florets may vary depending on cocksfoot cultivar and in this instance cv Akimidori produced nearly 40% more multiple seed units than cv Kara (Table 6).

Germination capacity is generally considered as one of the major criteria for judging the quality of seedlot. The cocksfoot cultivars observed here differ in their germination capability (Table 7) although overall germination ranged from 90-94 for single florets and 80-94 for multiple florets. Akimidori performed significantly better than the other 3 cultivars in both seed categories.

Table 7. Effect on % germination in single and multiple floret in different cultivars.

Variety	% Normal Seedling (Single floret)	% Normal Seedling (Multiple florets)
Wana	90.06 B	80.00 C
Kara	91.31 B	87.34 B
Makibamidori	91.22 B	87.97 B
Akimidori	93.91 A	94.13 A
LSD p<0.05	1.82	3.08

**** Means are average of 16 observations**

In the case of multiple florets the variation in germination capability was more obvious (Table 7). Again cv Akimidori significantly outperformed the other cultivars. About 15% higher germination was observed in cv Akimidori than in Wana with cv Makibamidori and Kara being similar and intermediate. Multiple floret germination was poorest in cv Wana. This suggests that although cv Wana has the highest pure seed yield, pure seed in cv Akimidori consisted of more viable seed units than in the other three cultivars.

4.2. Effects of pesticides

Efficacy of two different types of pesticide viz. nematicide (Vydate for controlling nematodes which have been implicated as possible vector for bacterial disease) and bactericide (Streptomycin for controlling bacterial pathogens) alone and in combination were tested against a traditional Folicur fungicide application. Differences between seed yield and other seed yield components occurred following the application of different pesticides. There was also some variation following seed cleaning particularly in pure seed content recovered from different machine separated fractions. These effects of pesticide on seed cleaning and seed recovery are presented in Section 4.2.2. Foliar disease as well as nematode populations were also influenced by different pesticide treatment and are presented in Section 4.2.3. Differences in seed quality due to pesticide treatment were small and are presented in Section 4.2.4.

4.2.1. Seed yield and yield components

Seed yield

The influence of four pesticide applications on harvested yield (g/m^2) showed noticeable variation between treatments (Table 8). The total amount of harvested seed produced in Vydate, fungicide or Vydate+Streptomycin (V+S) treated plots were all similar but produced significantly higher amounts of seed than in plots treated with Streptomycin alone (Table 8). The seed yield obtained in V+S, Vydate or fungicide treated plots were 34.3, 27.1 and 24.7% higher respectively than in Streptomycin treated plots. The data suggests that Streptomycin may have a depressing effect on seed yield.

Table 8. Effect of pesticide application on seed yield (g/m^2) of cocksfoot.

Pesticides	Harvested Seed Yield g/m^2	Pure Seed Yield g/m^2	% of Pure Seed Recovered
Fungicide (Control)	84.34 A	54.55 A	62.09
Vydate (V)	87.05 A	60.44 A	65.61
Streptomycin (S)	63.45 B	41.38 B	62.96
Combination (V+S)	96.39 A	60.99 A	61.55
LSD $p < 0.05$	18.49	12.92	NS

**** Means are average of 16 observations**

The influence of pesticide treatments on pure seed yield (g/m^2) are presented in Table 8. The data shows that the pure seed yield as well as harvested seed yield per unit area was only affected by Streptomycin application (Table 8). Vydate and fungicide alone or in combination had no effect on seed yield in cocksfoot, while Streptomycin had a negative and depressing effect on pure seed yield. Since fungicide was sprayed on all plots, the non significant additional benefit obtained with Vydate and V+S treatment clearly indicates that fungicide alone can be used in cocksfoot seed crops rather than in combination with a bactericide or a nematicide. The data on % recovery of pure seed was shown to be less in the Streptomycin treatment (Table 8) strongly suggesting the depressing effect on seed yield than any other pesticides.

Yield components

Variability in yield components due to different pesticide treatment were noticed in fertile tiller/m², number of spikelets/ head and in total floret numbers (Table 9). Seed weight and number of florets/spikelets were not affected by pesticide application (Table 9).

The fertile tiller number/m² was found to be differ following pesticide application (Table 9). Significantly higher and similar numbers of fertile tillers were recorded in the Vydate, fungicide and V+S treatments which were 15-20 % higher than that in the Streptomycin treatment. Significantly lower numbers of tillers in Streptomycin treated plots as compared to those treated with Vydate and V+S suggests that Streptomycin reduces fertile tiller number and have a negative influence on fertile numbers.

Table 9. Effect of pesticides application on seed yield components.

Pesticides used	Fertile tiller/m ²	Spikelets/head	Florets/spikelets	No florets/head	TSW
Fungicide (Control)	599.75 AB	321.10 B	4.61	1472.49 B	0.106
Vydate	644.50 A	365.08 AB	4.61	1675.54 A	0.105
Streptomycin	518.75 B	345.45 AB	4.62	1596.80 AB	0.107
V+S	604.62 A	368.54 A	4.58	1683.96 A	0.104
LSD p<0.05	85.35	43.06	NS	193.31	NS

** Means are average of 16 observations

Pesticide treatment however, did influence the total number of spikelets present per seed head (Table 9). Significantly higher number of spikelets/head was observed in V+S than in fungicide treatment. However, the differences in spikelets between fungicide, Streptomycin and Vydate was not significant.

The number of florets/head was also influenced by pesticide applications (Table 9). Significantly higher numbers of florets were observed in Vydate and V+S treatments than that in fungicide treatment. However no difference was observed between fungicide

and Streptomycin and the difference between Vydate, Streptomycin and V+S treatments were not significant. The effects of V+S over fungicide simply reflects differences in spikelet numbers since no difference in florets/spikelet.

Relationship between seed yield and different yield components with pesticides.

Linear correlation coefficients were calculated to determine if yield components were related to seed yield (Table 10). A significant and positive coefficient was obtained in fertile tiller number with all pesticides. However, the coefficient of correlation was highly significant (0.01) with fungicide, Vydate and V+S treatment. Although a positive correlation exists with Streptomycin the relationship is not significant. The TSW and number of florets/spikelet was negatively correlated with seed yield in all pesticide treatment. A positive and significant correlation was also obtained with number of spikelets/head in all treatments, but was highest in the fungicide and Streptomycin treatments. Since the coefficient of correlation in terms of tiller numbers is higher in the fungicide treatment followed by V+S and Vydate. These suggests that it is the number of reproductive tillers at anthesis, not florets/spikelet or TSW which was most seriously altered by pesticide application and which have a significant influence in determining seed yield.

Table 10. Simple correlation coefficient for yield against yield components with four pesticides.

	Fungicides	Vydate	Streptomycin	V+S
TSW	-0.621	-0.065	-0.497	-0.267
Floret/spikelets	-0.218	-0.415	0.191	-0.090
Spikelets/head	0.738*	0.513*	0.756*	0.552*
Florets/head	0.587*	0.525*	0.789*	0.355
Fertile tiller	0.859**	0.542*	0.115	0.714*

* Significance at 15 df.

4.2.2. Seed cleaning and seed recovery

Investigation on pure seed yield per unit plot from machine cleaned seed, screen fraction and blow fraction revealed that a considerable amount of pure seed of cocksfoot may be

lost in the cleaning process and pesticide may influence the proportion of such loss (Table 11). It was observed that only the Vydate and V+S improved pure seed recovery following machine cleaning compared to fungicide and Streptomycin. The seed yield in the V+S treatment is 34.2% higher than in Streptomycin treated plots. This suggests that the combination treatment of V+S can improve pure seed yield in machine cleaned seed while the remaining treatments are ineffective. The total weight of clean sample was also highest in V+S treatment however no difference with Vydate treatment (Table 11) suggesting Vydate may have removes the depression caused by Streptomycin.

Table 11. Effect of pesticide application on pure seed yield from commercial cleaning.

Pesticide	Total sample g/m ² (clean fraction)	Pure Seed yield g/m ² (Clean fraction) ¹	Total sample g/m ² (Screen fraction)	Pure Seed yield g/m ² (Screen fraction) ¹	Total sample g/m ² (Blow fraction)	Pure Seed yield g/m ² (Blow fraction) ¹
Fungicide (Control)	28.10 B	19.97 B (67.61)	19.59	12.79 (62.34)	36.65 A	21.79 (59.71)
Vydate	33.97 AB	24.59 AB (72.51)	21.33	15.40 (67.57)	31.74 AB	20.46 (64.01)
Streptomycin	25.39 B	18.96 B (71.97)	17.34	11.27 (61.68)	20.69 B	11.81 (57.00)
V+S	40.17 A	28.83 A (70.18)	17.39	11.70 (61.46)	38.83 A	20.77 (52.58)
LSD p<0.05	10.66	6.67	NS	NS	14.10	NS

¹ Figure in parenthesis indicates % purity

** Means are average of 16 observations

The total pure seed yield recovered from the screen and blow fractions was not affected by pesticide application. Similarly the total weight of the screen sample was also not affected by pesticide application.

The total weight recovered from the blow fraction showed significant variation due to pesticide treatment (Table 11) being lowest in Streptomycin than in fungicide and V+S treatment.

4.2.3. Disease and nematode population

Koch's Postulates studies were carried out in the glass house with pot grown plants being sprayed with a suspension containing nematodes isolated from field grown leaf samples with freshly prepared bacterial culture (Plate 4) or with a bacterial suspension prepared from an authenticated culture of *C. rathayi* (Plate 5) obtained from (ICMP). Inoculation was made at the time of floral initiation. None of these inoculated plants produced any visible symptoms on leaves or seed heads. This suggests that nematodes (Plate 6) are not a vector for *C. Rathayi* and hence may not be the problem in seed production in this instance. Although the colony colour of the isolated bacteria appeared similar to the authenticated culture, its failure of grow on diagnostic media (TTC) suggested that the bacterium consistently isolated from cocksfoot seed was not a *Clavibactor* sp., but most probably a saprophyte which did not produce the head distortion symptoms characteristic of *Clavibactor rathayi*.

The influence of different pesticide treatments on the nematode population revealed that the nematicide Vydate alone and also when used with Streptomycin reduced nematode populations to a greater extent than fungicide and Streptomycin application (Table 12). Vydate, however, was only effective in reducing nematode population by approximately 50% compared to levels in fungicide and Streptomycin treated plots. The nematode population recorded before applying pesticide showed only minor variation in number (23-27) suggesting that nematodes were uniformly distributed throughout the experimental site. This suggests that Vydate has a significant effect in reducing nematode populations when used alone or in combination with Streptomycin but does not eliminate nematodes.

Table 12. Effect of pesticide application on foliar disease incidence (1-5) and nematode population/4g leaf.

Pesticide	Disease incidence			Nematode population	
	Vegetative Growth stages	Peak flowering	Maturity (harvest)	Before Application	After Application
Fungicide (Control)	1	1	1.00 B	22.84	36.94 A
Vydate	1	1	1.25 A	23.94	18.88 B
Streptomycin	1	1	1.12 AB	25.00	39.31 A
V+S	1	1	1.00 B	27.56	19.31 B
LSD p<0.05	NS	NS	0.21	NS	5.04

**** Means are average of 16 observations**

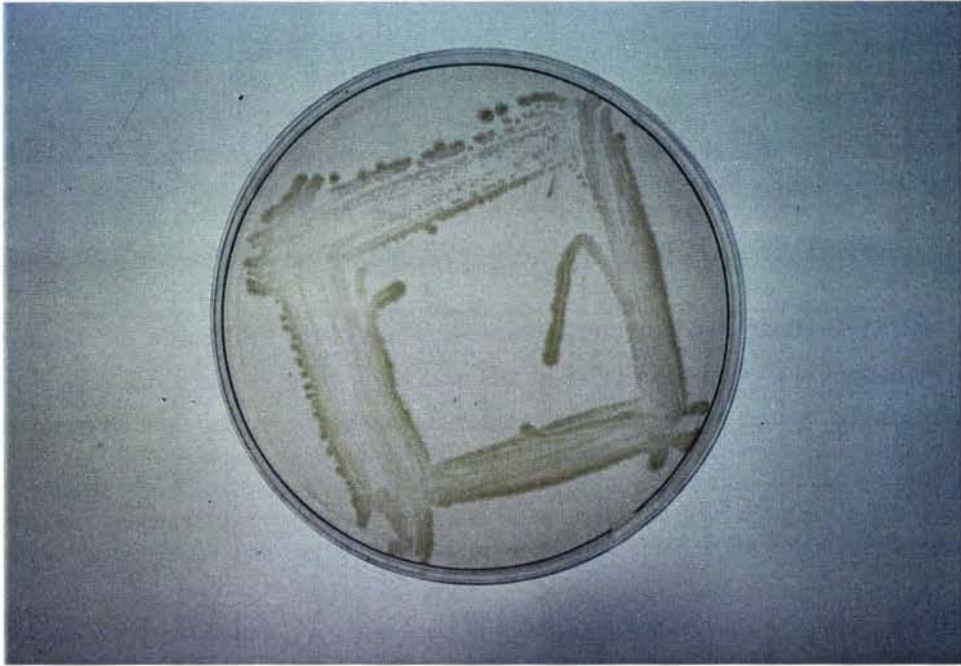


Plate 4. Bacterial culture from field grown cocksfoot

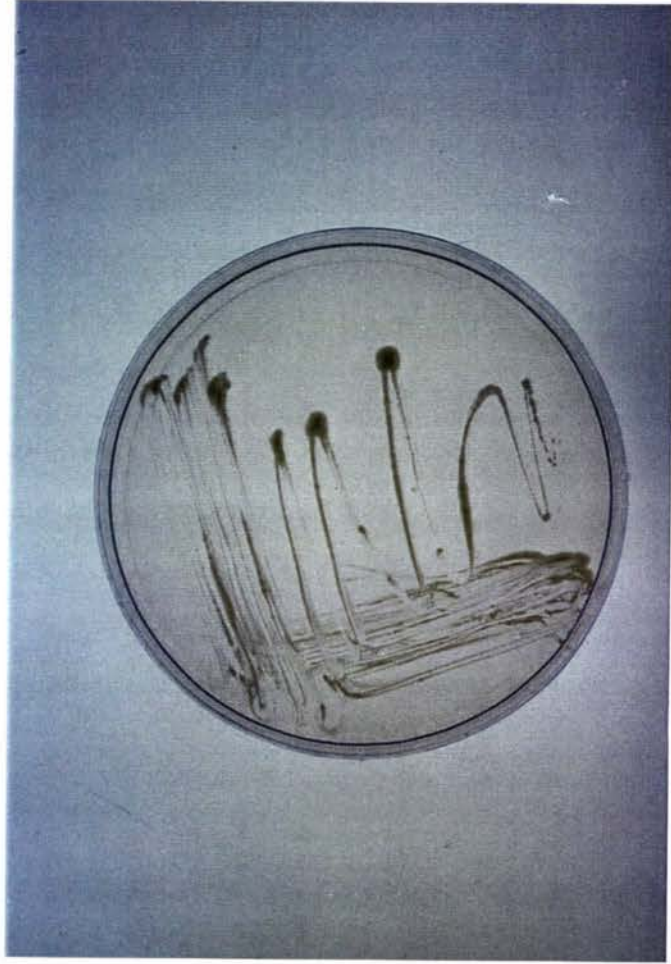


Plate 5. Authenticated culture of *C. rathayi* (from ICMP)

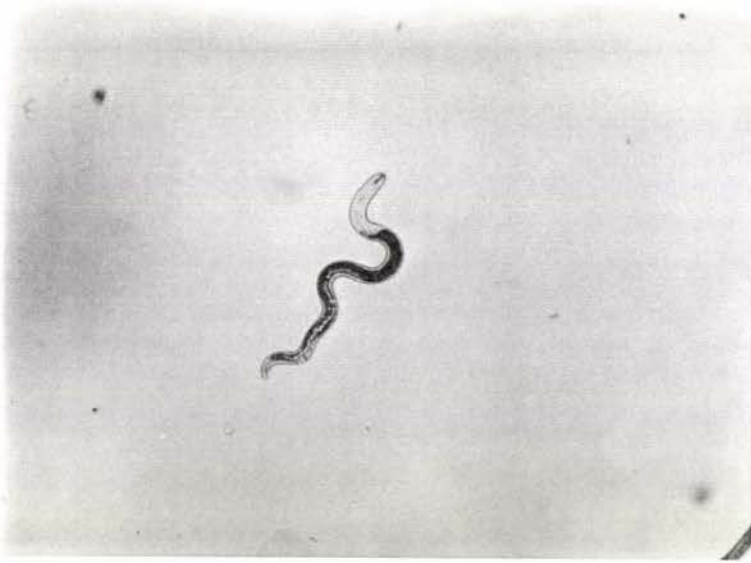
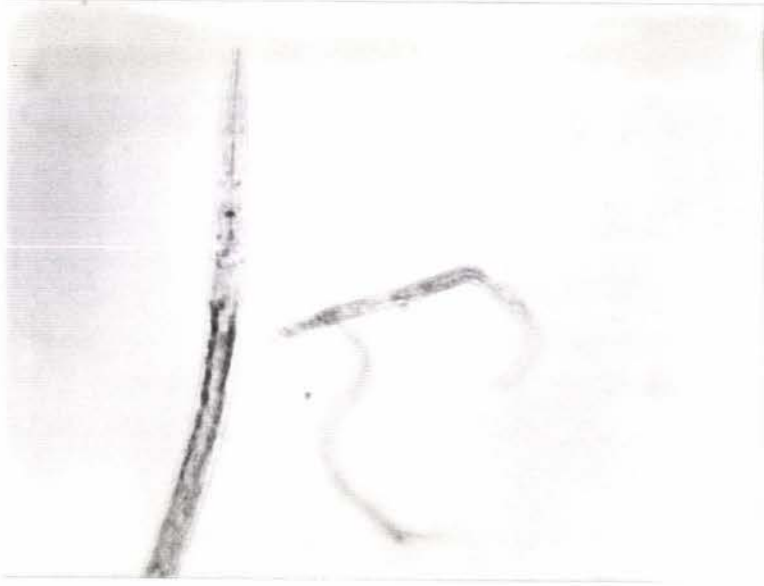


Plate 6. Nematodes isolated from cocksfoot fields

The incidence of foliar disease (eye spot Plate 7) visually recorded on a 1-5 scale during the experiment was very low during vegetative growth stage, at peak flowering and at seed maturity in all pesticide treated plots (Table 12). Although, disease severity in this experiment varies significantly at harvest (maturity) but did not increase seed yield as a result. However, the agronomic implication of a 20% higher natural disease incidence in Vydate treated plots than in fungicides is likely to be low. Under higher disease pressure, however, it is possible that fungicide may be more effective in reducing foliar disease severity in cocksfoot.

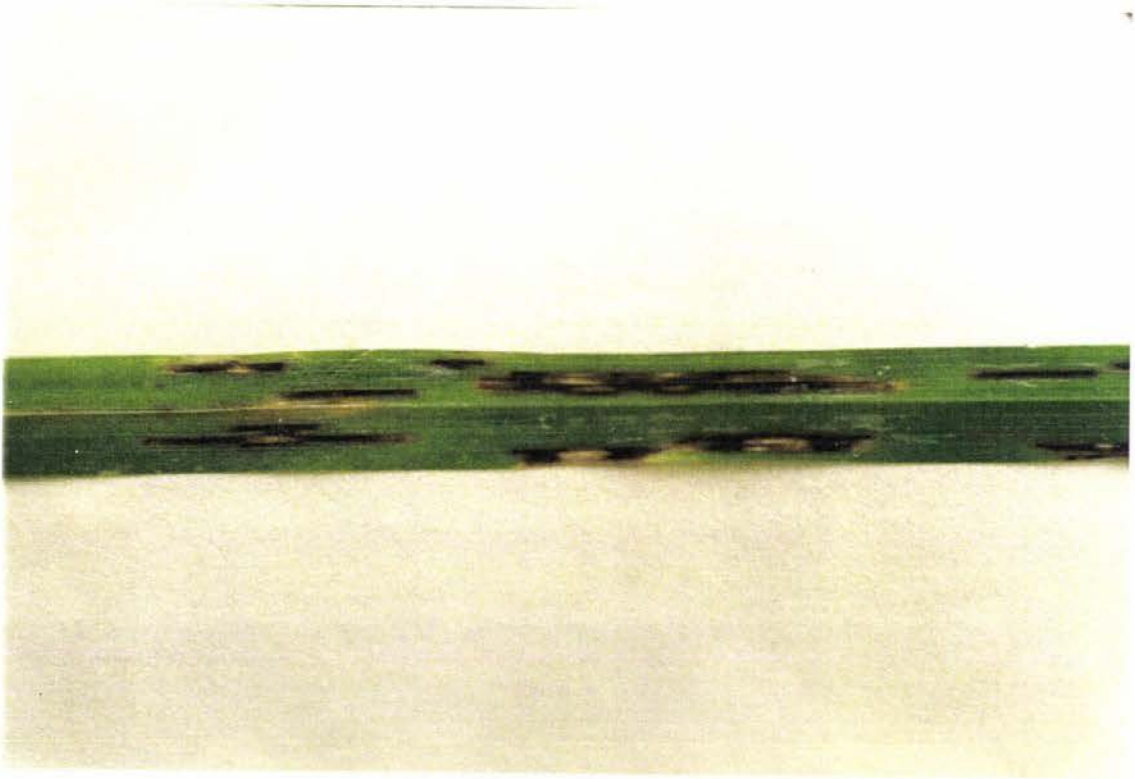


Plate 7. Eye spot disease in cocksfoot

4.2.4. Seed quality

Pesticide treatment had no major influence on seed quality in cocksfoot. In the machine clean seed and in the blow fraction material the percentage of pure seed was unaffected by pesticide treatment (Table 13). Similarly in the screen fraction, although the seed purity varied statistically (Table 13) been higher in Vydate treatment, this effect is unlikely to be of any obvious agronomic or commercial importance.

Table 13. Effect of pesticide application on seed quality

Pesticide	% purity in clean fraction	% purity in screen fraction	% purity in blow fraction
Fungicide (Control)	67.61	62.34 B	59.71
Vydate	72.51	67.57 A	54.01
Streptomycin	71.97	61.68 B	57.00
V+S	70.18	61.46 B	52.58
LSD p<0.05	NS	4.95	NS

**** Means are average of 16 observations**

Pesticide treatment had a minor influence on the germination capacity of single florets of cocksfoot (Table 14). All single floret germination results exceed 90% with multiple floret germination being consistently above 85%. The proportion of multiple floret units present in the pure seed were not influenced by any pesticide treatment (Table 14).

Table 14. Effect of pesticide treatment on germination and on proportion of multiple floret units.

Pesticide	Proportion of Multiple florets (%)	% Normal Seedling	
		Single florets	Multiple florets
Fungicide (Control)	20.83	90.06 B	87.25 AB
Vydate	21.96	92.47 A	86.63 B
Streptomycin	23.25	91.41 AB	86.47 B
V+S	23.89	92.59 A	90.09 A
LSD p<0.05	NS	1.81	3.08

**** Means are average of 16 observations**

4.3. Combined influence of cultivar and pesticides on seed yield

The combined cultivar and pesticides on seed yield and different yield components, seed cleaning, disease and on seed quality have already been investigated separately in the above sections. However the expression of seed yield, yield components and cleaning could not be completely explained without also considering their combined treatment effects. The effect of 4 cocksfoot cultivars in combination with 4 different pesticide treatments on seed yield, yield components, seed cleaning, disease severity and on seed quality are discussed below.

4.3.1. Seed yield and yield components

Seed yield

The results of individual treatments on seed yield are shown in Fig. 2. Considerable variation in seed yield (harvested yield/m², pure seed yield/m².) both within and between treatments occurred. Harvested seed yield varied from a lowest of 42.94g/m² in cv Akimidori combined with Vydate treatment to highest of 150.56g/m² in cv Wana (Fig 2 and Appendix 5) combined with fungicide treatment. Similar trends were also observed in pure seed yield/m² (Fig 2 and Appendix 5). There were very highly significant effects due to cultivar (Appendix 6) and a significant interaction (0.05) both for harvested and pure seed yield between cultivar and pesticide, with greatest response from cv Wana with fungicide combination. However, no differences were apparent in Wana + Vydate and Makibamidori + (S+V) combinations (Fig 2). The results strongly indicate that cultivar differences act more independently than pesticide.

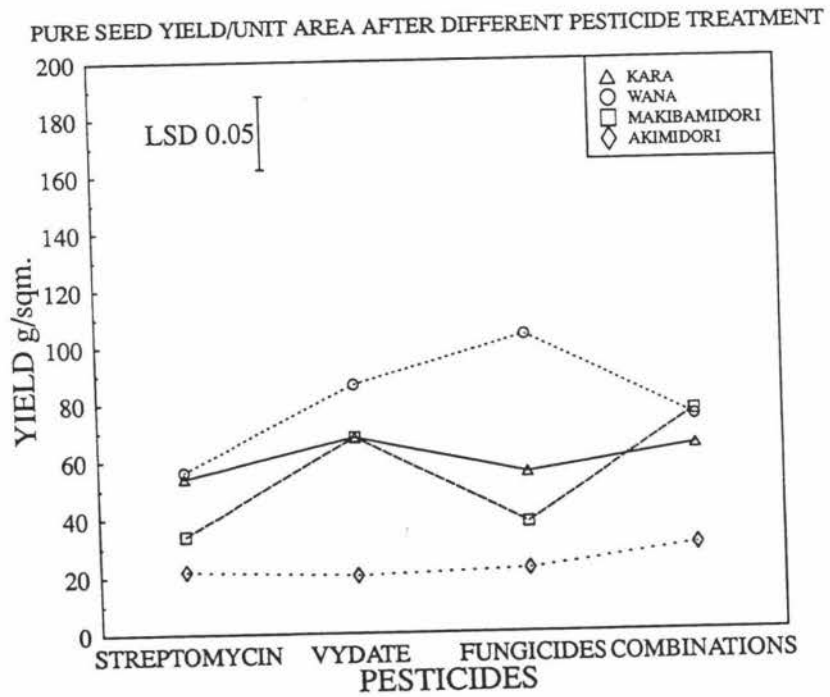
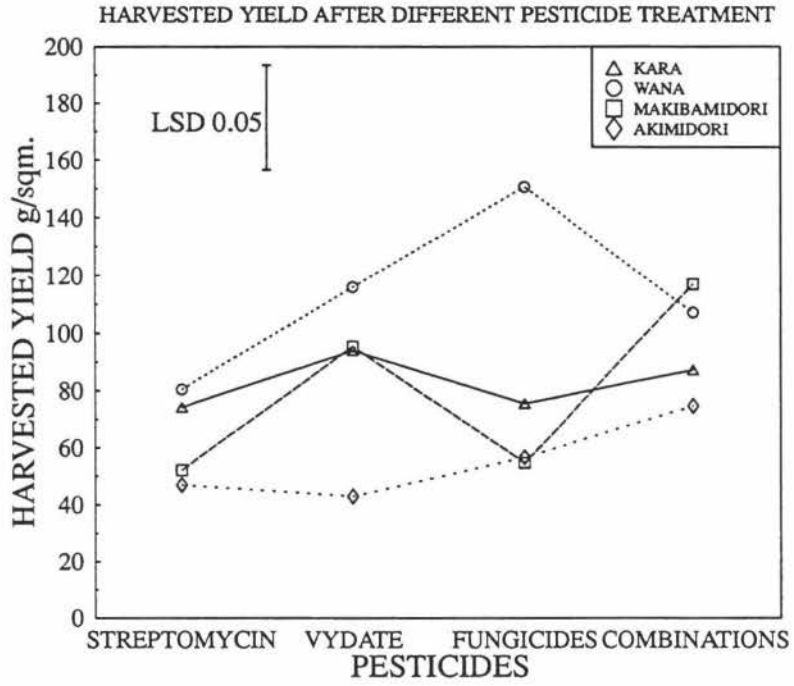


Figure 2. Seed yield in 4 cocksfoot cultivars under different treatment combinations.

Seed yield components

Among the seed yield components studied only fertile tiller numbers significantly interacted (Appendix 8) with cultivar and pesticide with greatest response in cv Wana plus with fungicide (Fig. 3 & Appendix 7). However, no difference in Vydate treated plants in cvs Kara, or Wana, or in the Vydate plus Streptomycin treatment with cv Kara occurred. Results on fertile tiller number at anthesis indicates that the highest number of 827 tiller/m² was produced in cv Wana combination with fungicide compared with a lowest number of 475/m² in cv Akimidori with Vydate plus Streptomycin and in cv Makibamidori with Streptomycin combination (Appendix 7).

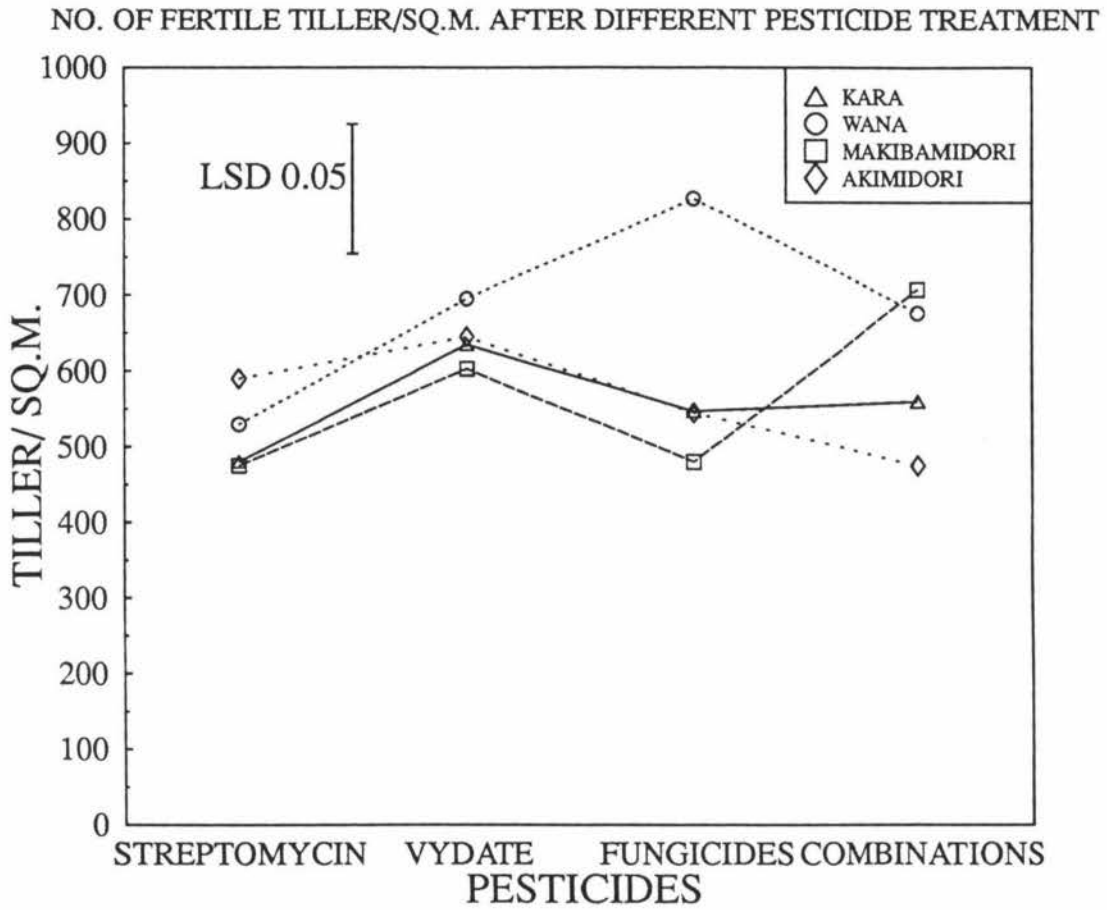
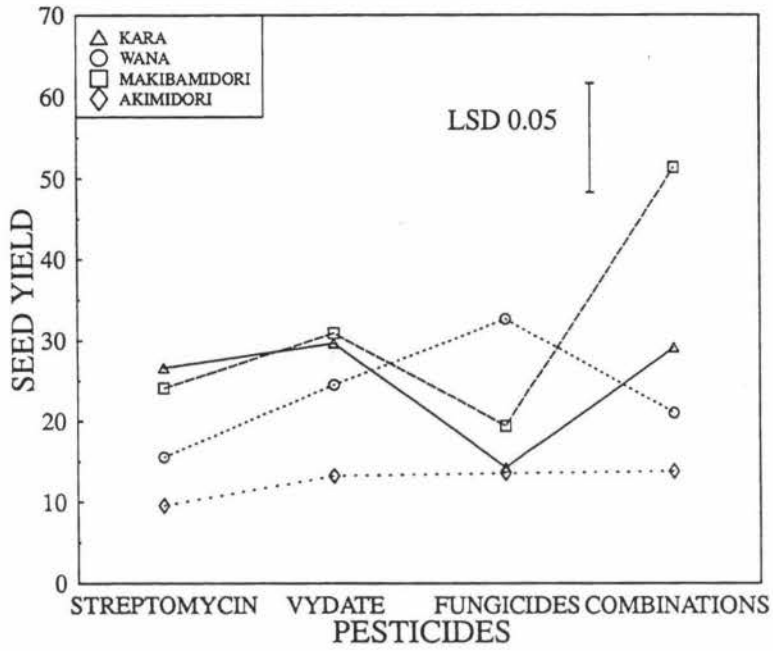


Figure 3. Fertile tiller production in 4 cocksfoot cultivars in different pesticide treatment combinations.

4.3.2. Seed cleaning and seed recovery

Pure seed yield from the clean fraction was highest in cv Makibamidori with V+S combination and lowest in cv Akimidori treated with Streptomycin (Fig. 4 and Appendix 9). However pure seed yield in V+S treatment with cvs Wana and Kara are similar. Although there was a highly significant (0.05) interaction between cultivar and pesticide (Appendix 10) the very highly significant effect of cultivar (Appendix 10) strongly suggests its dominant role in pure seed recovery from the clean seed fraction. There was also a significant interaction between cultivar and pesticide for seed recovery from the screen fraction with the highest response from Streptomycin with cvs Kara and Wana, Vydate with cvs Kara and Wana, fungicide with cvs Kara, Wana and Makibamidori, and V+S with cv Akimidori. There was no interaction for pure seed recovery in the blow fractions (Appendix 10). The very highly significant effect of cultivar (0.01%) in all the three fraction and insignificant effect of pesticide in screen and blow fraction (Appendix 10) again indicates the dominance of cultivars on pure seed recovery in different fractions of seed.

PURE SEED YIELD FROM MACHINE CLEAN SEED AFTER DIFFERENT PESTICIDE TREATMENT



PURE SEED YIELD FROM SCREEN LEFTOVER AFTER DIFFERENT PESTICIDE TREATMENT

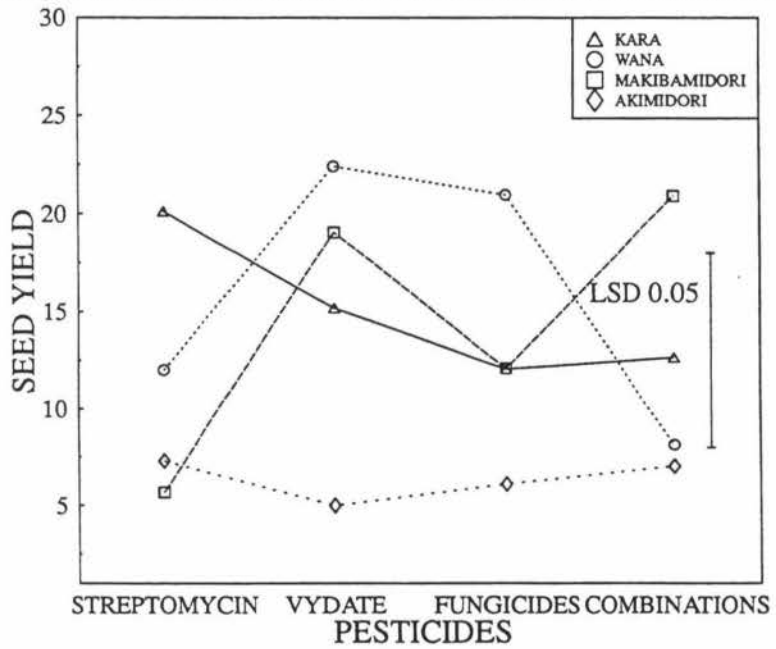


Figure 4. Pure seed yield recover from different fractions in 4 cocksfoot cultivars at different treatment combinations.

4.3.3. Disease and nematode population

There was no interaction between cultivars and pesticide for foliar disease incidence at harvest or between cultivar and nematode (vector) population before and after pesticide application (Appendix 12).

3.4. Seed quality

There was no interaction between cultivar and pesticide for percent purity in the clean seed fraction or in the screen fraction. In the blow fraction there was a significant interaction (Appendix 14). The highest response in blow fraction comes from Streptomycin in cvs Kara and Wana, Vydate in cvs Kara, Wana and Makibamidori, fungicide in cvs Kara, Wana and Makibamidori and V+S in Kara and Wana (Appendix 13). Lowest response was with cv Akimidori with all pesticides. Overall the germination of single florets exceeded 89% but there was no interaction between cultivar and pesticide (Appendix 11 & 14). However there was an interaction between pesticide and cultivar in terms of multiple floret percentage with lowest numbers in cv Wana for all pesticide. These highly significant effects of cultivar (Appendix 14) in relation to levels of purity in different cleaning fractions, germination of multiple florets and proportion of multiple florets strongly suggests that these parameters are mainly controlled by cultivar ie cultivar acts more independently than pesticides.

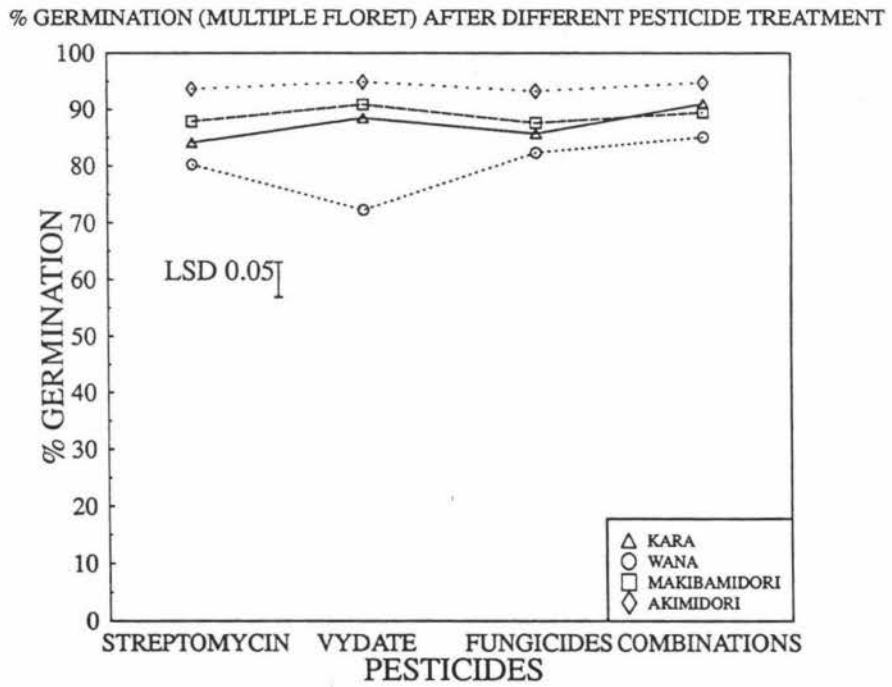
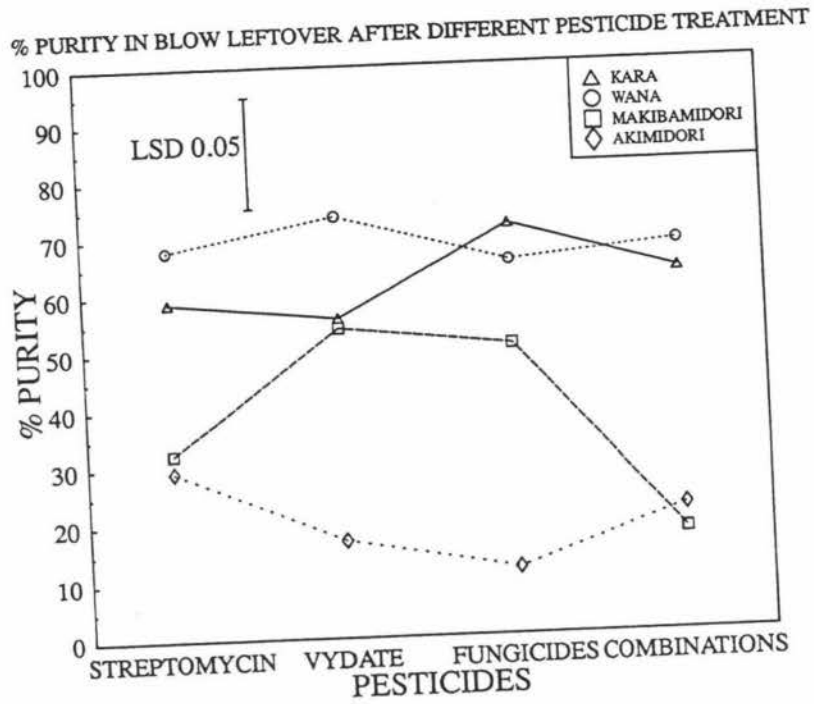


Fig. 4. Seed quality in 4 cocksfoot cultivar under different treatment combinations.

4. 4. Effect of fungicide in seed production of cocksfoot cv Tekapo (Trial 2)

The efficacy of three different fungicides was evaluated on cocksfoot cv Tekapo to determine their effectiveness or otherwise in influencing seed yield, seed yield components, disease severity and seed quality. Only minor effects on seed yield, yield components and on seed quality were observed.

4.4.1. Effect of fungicides on seed yield and yield components

The results of fungicide treatment revealed that none of the fungicides used improved seed yield compared to the control. The pure seed yield/m² in Bavistin treated plots was about 11, and 5 % higher than Alto and Bravo treated plots although the difference with control plants was not significant (Table 15). Similarly the harvested yield in Bavistin treated plot was approximately 25% higher than the control and 20 % , and 17% higher than Alto and Bravo treated plants one (Table 15). Again, however results were not significant.

Table 15. Effect of different fungicides on seed yield and yield components in cocksfoot cv Tekapo.

Treatment	Harvested yield/ m ² (g)	Pure seed yield/ m ² (g)	No. of spikelets /head	No of florets/ head	No of florets/ spikelets	Fertile tiller/ m ²	TSW
Alto	68.23	44.87	278.55	1255.9	4.71	675.00	0.105
Bavistin	91.54	50.56	291.50	1345.7	4.60	745.00	0.100
Control	76.03	42.82	265.75	1210.6	4.59	655.00	0.100
Bravo	73.36	48.08	234.55	1077.4	4.56	740.00	0.100
LSD p<0.05	NS	NS	NS	NS	NS	NS	NS
CV%	30.24	28.33	17.53	20.68	6.04	17.44	6.38

Although none of the fungicides tested increased seed yield or influenced seed yield components there were minor effects in their disease (eye spot) control effectiveness (Table 16). Foliar disease incidence was visually scored on a 1-5 rating scale at three different stages of crop growth viz. vegetative growth stage (17.11.94), peak flowering (1.12.94) and at harvest ripeness (23.12.95). Results are presented in Table 16. Although disease levels were initially low (1.5 - 1.75 disease score) fungicide application had some effects in reducing but not eliminating disease levels at harvest. In particular Alto

significantly reduced disease incidence by approximately 35% (2.75) compared with the control (sprayed with water only) treatment (4.25 relative disease score) at harvest. The other two chemicals (Bavistin and Bravo) were, less effective.

Table 16. Effect of different fungicides on disease incidence (1-5 scale) in cocksfoot cv Tekapo.

Treatment	Disease incidence (1-5 scale)		
	At the beginning	At peak flowering	At harvest (full maturity)
Alto	1.75	2.75	2.75 B
Bavistin	1.50	3.00	3.25 AB
Control	1.75	3.5	4.25 A
Bravo	1.75	2.75	3.00 AB
LSD $p < 0.05$	NS	NS	1.31
CV.%	14.81	17.57	24.78

4.4.2. Seed cleaning and seed recovery

Fungicide treatment did not influence pure seed recovery from any of the fractions (Table 17), probably suggesting fungicide application in cv Tekapo has no effect in improving seed recovery in machine cleaning however, with a very high level of coefficient of variation (cv%) in all the seed fractions (35-89). The total weight of sample in any fraction was also not affected by fungicide treatment.

Table 17. Effect of different fungicides on pure seed yield in g/m² from different fraction of seed in cocksfoot cv Tekapo.

Treatment	Total Weight g/m ² (clean fractions)	Pure seed yield g/m ² (clean fractions) ¹	Total weight g/m ² (screen fractions)	Pure seed yield g/m ² (screen fraction) ¹	Total weight g/m ² (blow fraction)	Pure seed yield g/m ² (blow fractions)
Alto	39.29	25.76 (64.64)	12.70	8.46 (64.78)	16.18	10.65 (58.16)
Bavistin	49.8	29.18 (61.36)	16.29	9.83 (57.89)	28.45	11.55 (36.87)
Control	36.2	24.18 (67.44)	10.89	7.27 (66.75)	28.94	11.38 (35.90)
Bravo	38.86	26.49 (68.91)	14.2	8.46 (60.28)	20.31	13.09 (57.9)
LSD $p < 0.05$	NS	NS	NS	NS	NS	NS
CV%	53.42	50.75	35.26	34.52	66.67	88.85

¹ Figure in the parenthesis indicate % purity

4.4.3. Seed quality

The germination results in single and in multiple florets were found to vary significantly between fungicide treatments (Table 18). In single floret test significantly higher germination was recorded in Bavistin compared to control while other are ineffective in improving gemination. Similarly in multiple floret test higher germination was also recorded in Bavistin and Bravo treated plants compared to control. This suggests that these two fungicides may improve germination particularly in single florets although the overall gemination recorded here is well above the commercially accepted level (85%+).

Table 18. Effect of different fungicides on % germination in single and multiple floret, percentage of multiple floret unit and % purity in different fraction of cocksfoot cv Tekapo.

Treatments	% purity in clean seed	% purity in screen fraction	% purity in blow fraction	% Multiple seed in pure Seed	Germination	
					Single floret	Multiple floret
Alto	64.64	64.78	58.16 A	22.78	88.63 B	86.50 AB
Bavistin	61.36	57.89	36.87 C	25.65	96.25 A	89.38 A
Control	67.44	66.75	35.90 C	25.24	89.63 B	80.63 B
Bravo	68.91	60.28	57.9 AB	25.05	92.63 AB	90.50 A
LSD p<0.05	NS	NS	20.37	NS	4.14	6.82
CV%	12.79	11.27	27.08	17.85	2.82	4.92

CHAPTER V

DISCUSSION

Effect of cultivar on seed yield and yield component (Trial 1)

This field trial with 4 cocksfoot cultivars as a 3 year old stand revealed that the New Zealand cultivar Wana significantly outyielded the remaining three cultivars. Cultivars Kara and Makibamidori performed equally well and cv Akimidori was ranked lowest in terms of seed production. About 70% higher pure seed yield was recorded in cv Wana compared to the lowest yielder (Akimidori) while cv Kara and Makibamidori produced 33% and 30% more seed respectively than cv Akimidori (Table 2). Although the seed yield in each cultivar followed a similar pattern to the first year work by Wilson (1995) on the same crop area, the pure seed yield in general was low in the third year compared to 1st and 2nd year results and about 35%, 11%, and 28% less pure seed was observed in cvs Wana, Kara, and Akimidori respectively. The seed yield obtained in cv Makibamidori was higher than Wilson's (1995) 1st year results and about 25% higher yield was observed from her spring sown trial in the presence of fungicide. Martyniak and Domanski (1984) also noticed that seed yield stability is often decreased with stand age. Niemelanen (1991 b) observed similar variation in seed yield of 5 cocksfoot cultivars during 1977-1990 in Finland and found that second year stands produced higher seed yields compared with first and third year stands. The reason for these yield differences was mainly due to inadequate floral evocation (induction), and in some instances high winter plant damage and pre harvest shattering. Johnston (1960) also noticed in cocksfoot that some unknown factor, apart from pest damage, has a marked effect on the percentage of hermaphrodite florets that fail to produce fully developed caryopses resulting in large (45-65.9%) percentages of florets being sterile and incapable of caryopsis development under any conditions. Similarly Stapledon and Wheeler (1954) found that decline in seed yield from year to year in cocksfoot stands, although alleviated to some extent by N fertilizer aftercare, was common in cv S 143. The significantly higher seed yield of cv Wana comes mainly from the significantly higher number of fertile tillers/m² (Table 3) and higher numbers of florets/head while cv Kara seed was significantly heavier than the seed of the others. Similarly, the higher seed yield in cv Makibamidori compared to Akimidori is mainly due to heavier seed weight.

The poor performance in terms of seed yield in cv Akimidori may be related to its abortion of florets (poor floret site utilization). Despite this, its earliness in flowering still allowed the production of similar numbers of tillers at anthesis, spikelets/head and florets/head compared to cv Makibamidori. The higher yield in cv Wana mostly came from its higher number of fertile tillers. Similar opinion has been expressed by other investigators who have clearly shown that fertile tiller numbers have the main effect on seed yield in cocksfoot and other grasses (Langer 1980). The significantly high positive coefficient of correlation value (Table 4) obtained with cv Wana (0.916) in this study supports the impact of fertile tiller numbers on seed yield. With cv Akimidori, a positive but nonsignificant coefficient of correlation clearly indicates that production of lower fertile tillers/m² (Table 3) is one of the main reasons for low yield in this cultivar. In the two Japanese cultivars, both showed similar numbers of spikelets/head, florets/spikelets, total florets/head and fertile tillers/m². However seed weight (Table 2) was significantly higher in cv Makibamidori which outyielded Akimidori by 30% (seed yield). This suggests that the higher seed yield in cv Makibamidori was due mainly to its higher seed weight (TSW) although its flowering time (mid November) was almost one month later than cv Akimidori. The two New Zealand cultivars also reached peak flowering in mid November. Weather data indicates that the mean temperature in October was less (15.6 max. 11.8 min.) than in November (22.4 max. 14.1 min.) (Appendix 2), which may influence seed set in the early flowering cultivar Akimidori. Moreover in October 1994 the minimum grass temperature was below freezing (0° C) on 7 occasions with a monthly mean of 4.0° C. In November only two ground frosts were recorded and the mean (monthly) temperature was 7.3° C (Appendix 3).

The lower seed yield in cv Akimidori in this study may also be due to unsuitable weather conditions. Firstly, floral induction in cocksfoot is a chemical or hormonal differentiation resulting from the fulfilment of certain thermo-photoperiodic requirements (Gardner and Loomis, 1953). Cocksfoot has a juvenile stage where vegetative growth continues but the plant is not induced to flower in conditions which later induce flowering (Bernier *et al.*, 1981; Calder, 1963 and 1964a; Cooper and Calder 1964; Heide 1987). The duration of the juvenile stage has been estimated to last from 4 to 7 weeks from sowing (Calder 1963 and 1964a; Heide 1987) or until a certain number of leaves

have developed on the plant (Broue 1973; Ikegaya *et al.* 1981; Blondon 1985). Variation in the flowering requirements of cocksfoot cultivar is common (Calder 1964b). Early plant breeders (eg. Stapledon 1928) were aware that differences in growth between varieties at certain times of the year were a function of their earliness or lateness which depends on the number of tillers that become reproductive, and the photoperiodic factors that influence the process in different genotypes (Langer 1958). Gardner and Loomis (1953) suggest that induction requires a photoperiod of the order of 12 hours or less, either accompanied or immediately followed by air temperatures between 5 and 10°C. The initiation of floral development requires warmer temperatures and longer days. Normal development of the panicle requires a photoperiod of more than 13 hours and Calder, (1964b) noticed that daily exposure to seven hours of darkness is near the minimum for induction. Recently Heide (1987) reported in cocksfoot that the initiation of floral primordia did not take place in shortdays but required a transition from SD to LD with a critical photoperiod for secondary induction of about 12-13 h, depending on the origin of the cultivar and a critical number of 12 to 16 LD cycles was required for 100 % flowering (a reproductive tiller initiate flowers). The inability of cv Akimidori to respond fully to the conditions at Aorangi may have been a response to unsuitable daylength and/or temperature conditions. The lower number of spikelets as well as the total number of florets per head (Table 3) as observed in the present study with cv Akimidori was possibly related to this photoperiodic effect as mentioned by Heide (1987).

The interaction between temperature and photoperiod is also important for flowering in cocksfoot. It has been suggested that the effect of low temperature on flowering in cocksfoot populations is confined to the terminal LD treatment (Broue and Nichollst 1973). Flowering response also depends on the duration of SD vernalization and on temperature level of the subsequent LD eg. Niemelainen (1990) found 16h day length was the best for floral initiation and for culm elongation but the critical day length for culm elongation was between 12 and 14h (Niemelainen 1990). Seed yield differences in different genotypes due to timing of the reproductive phase and stem elongation is common (Knight 1963). The average daylength data for Palmerston North (Gordon pers. comm.) shows that day length in the mid October to mid November ranges around 12.5

compared with 14h and longer from mid November (Appendix 1). The mean radiation data in the experimental site also indicates lower radiation in October (22.4 MJ) compared to (25 MJ) in November (Appendix 2). This, perhaps, suggests that the relatively poor performance of the Japanese cultivar Akimidori in terms of seed yielding capability may be related to inadequate day length (photoperiodic requirement) at the time of flower initiation as has been suggested by Heide (1987); Niemelainen (1990) and Wilson and Thomas (1971). The results of this study also agree with findings by Calder (1964b) that much of the variation in flowering requirement in cocksfoot populations is associated with the place and latitude of origin of the plant material.

Frost damage may also be one of the causes of lower seed yield in cv Akimidori as reported by many researchers (eg. Niemelainen 1991a; Nordestgaard, 1982). Frost occurrence after floral initiation can decrease the number of panicles in cocksfoot (Niemelainen 1991a) and a -6°C frost at the flowering phase has been reported to destroy all caryopses (Nikolaeskayas, 1973) and that damage is specific to flower primordia (Heide 1980). Similarly, a two degree frost during the first 4 days of seed development has been shown to reduce seed yield in perennial ryegrass (Hill, 1971). Jiro (1989) from Hokkaido (Japan) tested 16 cocksfoot cultivars including cv Akimidori and produced a wintering index (wi) reflecting the winter ability of the different cultivar derived from different countries of origin. He ranked cv Akimidori (66 wi) as lowest compared to cultivars from Finland (124 wi), Canada (110 wi) and the USA (105 wi). It is therefore suggested that the lower yield in this cultivar (Akimidori) compared to the New Zealand cultivars and Makibamidori probably related to the low temperatures recorded during late flowering and early seed development stage (Appendix 3).

In spite of the different latitudes Japan (28°N - 45.30°N) and New Zealand (34°S - 48°S), similar seed yields were recorded from the Japanese cultivar Makibamidori and the New Zealand cultivar Kara. This may be due to similarity in yield components except total numbers of florets/seed head (Table 3). However the significantly higher number of florets/head in cv Kara failed to produce significantly higher seed yield possibly due to more abortion as evident in greater seed loss in the cleaning processes especially in the "blow fraction" of seed (Table 4). By comparison, cv Makibamidori produced higher

pure seed yield because its "clean seed" fraction contained more pure seed than other cultivars. This confirms the results of Brown (1981) who found that the larger the floret population, the lower the number of seeds obtained from every 100 florets in cocksfoot (cv G17). The higher yield from cv Makibamidori compared to Akimidori in this experiment also agrees with first year findings by Wilson (1995) but disagree with her second year results where she obtained better pure seed yield in cv Akimidori than in cv Makibamidori. This may be due to more favourable weather as cv Makibamidori flowers at a similar time to cvs Wana and Kara. In Wilson's second year trial tiller number in cv Makibamidori was not similar to either of the New Zealand cultivars although she found cv Makibamidori to be the only cultivar which showed a significant association between tiller number and seed yield, with highest seed purity. Hampton and Hebblethwaite (1983b) considered that fertile tiller numbers are not usually limiting unless there is poor establishment or moisture stress. The higher yield in cv Makibamidori in this third year trial might be due to production of more fertile tillers.

The lower seed yield in this trial in all cultivars compared to the 2nd year yield obtained by Wilson (1995) as well as the higher yield observed by the researcher may also be related to shattering. In cocksfoot and in many other forage grasses, seeds do not mature and ripen uniformly because of a protracted flowering period (McWilliam 1980; Young, 1991). Consequently early maturing seeds frequently shatter before late developing ones mature (Bean, 1965; Griffith, Lewis and Bean, 1980; Wilkins, 1991). The observed lower seed yields obtained in this trial compared to those obtained in the same site is possibly related to this shattering problem. Normally maximum yields of viable seed of commercial cultivars of cocksfoot are obtained at the "cream cheese" stage (Pegler, 1976), which corresponds to about 40% moisture content (Klein and Harmond, 1971). Around 20- 33% loss of potential seed yield in cocksfoot due to delayed harvesting has been recorded (Pegler 1976, and Nellist and Rees 1963, Andersen and Andersen, 1980). However, there are reports of non shattering Italian cocksfoot cultivars (Falcinelli *et al*, 1984) where shattering is under genetic control with a high degree of heritability (Falcinelli *et al*. 1989). An important observation in this study noted during anthesis was the period over which seed heads flowered. In this trial there were still heads flowering just prior to harvest. Meiger (1985) suggested that uneven ripening was a major factor

contributing to poor utilization of yield potential in perennial ryegrass. Because all reproductive tillers in cocksfoot do not initiate flowers at the same time this results in differences in seed head maturity. When harvest date is determined, the moisture content is measured from a range of seed heads in which seed is at a range of moisture levels. Harvest will therefore tend to take place at a time when only a majority of seed heads are ripe. At this time some seed will have started shattering and other seed will be immature. Although data on losses due to seed shattering were not recorded in the present study, the seed yield data (Table 2) plus the poor yields recorded in previous years, by different cocksfoot seed growers in the Canterbury area despite adequate management, suggest that lower yield may possibly be related to seed shattering.

Effect on disease severity

The severity of foliar disease (eye spot) recorded in all cultivars was very low (Appendix 4). Similar observations were recorded by Wilson (1995) from the same plots (< 1% eye spot and <5% rust) in the previous year (1993). This suggests that all cultivars were equally unaffected by eye spot disease. It is important to note that over the period of reproductive development, weather conditions were not very conducive to high disease infection because of low rainfall during September and October 1994 (Appendix 2) but were good for reproductive development. In general plant pathogens tend to require warm temperatures and the presence of moisture to become active following winter and early spring (Agrios, 1969). The susceptibility of a cultivar to a particular pathogen depends on weather conditions, availability of sufficient inoculum and on genetic characteristics. The incidence of foliar disease in cv Tekapo of trial 2 was high with plenty of rust inoculum also being available in surrounding fields. Despite this results in the present trial, the natural incidence eyespot disease was also low (<1%) even in the absence of fungicide in these four cultivars (Wilson, 1995) in previous year.

There were four genera of nematodes, mostly free living and fungal feeding species isolated from leaf tissue (Appendix 15) and seven genera from soil (Appendix 16) of which *Pratylenchus* and *Helicotylenchus* are considered to be pathogenic. However, absence of the genus *Anguina* (which is known to transmit *Clavibactor* sp in wheat) and failure to duplicate disease symptoms on cocksfoot plants inoculated with an

authenticated culture of *C. rathayi* strongly suggest that Rathay's disease may not be the problem and not transmitted by these nematodes. From the literature it was suggested that nematode control might be important in preventing Rathay's disease in cocksfoot. Despite the fact that the nematodes present in the crop were uniformly distributed throughout the trial site and the moderate control of nematodes number by the use of Vydate, no evidence of Rathay's disease in the field or following inoculation of glasshouse grown plants with a suspension of nematodes obtained from the field.

Effect of seed cleaning

The greater recovery of pure seed from the "blow fraction" of cv Wana compared with other cultivars suggests it is more susceptible to cleaning losses than the other three (Table 5). In contrast, cv Akimidori appeared to be less susceptible to cleaning losses. The often significantly lower seed recovery in Akimidori in all cleaning fractions (Table 5) as well as less production of harvested material indicates its unsuitability for commercial seed production in this climate. Similarly the lower pure seed recovery in this cultivar (Table 2) indicates the production of more empty florets and suggest the possibility of abortion during flowering or during early seed development. The higher losses of pure seed in cv Wana as observed here were related to lower seed weight in this cultivar. Pure seed yield losses in machine cleaning is common and well documented. Harmond *et al.*, (1968) observed cleaning losses in grass seed and reported that the procedures used to meet quality standards may result in a loss of upto 50% of good seed even through specialist machines and techniques are used for seed cleaning and handling.

Seed quality

Observations at the Official Seed Testing Station, Palmerston North, New Zealand have suggested that seedlots of cocksfoot and some other grasses often contain immature seed (Scott and Hampton, 1985). The variation in TSW within the cultivars used in this study appear to be mostly affected by genotype. Wilson (1995) also recorded significant variation in TSW between these four cultivars. Similarly Rumball (1982b) also noticed lower TSW in cv Wana compared to Kara. The germination in single florets and multiple florets was also found to be vary significantly between cultivars, with higher

germination being also observed in cv Akimidori. This suggests that pure seed of cv Akimidori consists of more viable seed than in other cultivars. The lower germination in multiple florets compared to single florets also suggests that seed lots containing higher percentages of multiple florets are likely to also have lower germination than seedlots containing a predominance of single florets. This result supports similar findings by Charlton and Thom (1984) and Scott and Hampton, (1984) who showed that seedlot germination could be increased by reducing the percentage of multiple seed units.

Effect of pesticides:

In terms of the effect of different pesticide application on seed yield, only Streptomycin appeared to have an adverse affect on pure seed yield (Table 8). This was considered to be a phytotoxic effect as previously identified (Anon. 1976). Because of its contact action the usually recommended spraying frequency for Streptomycin (7 day intervals) in this study was greater than for the other pesticides used. This is in agreement with findings by Putten *et al* 1990, Ingham *et al* 1991) who recorded a similar deleterious effect of Streptomycin on growth in other grasses (eg. marram grass/ *Ammophila arenaria*).

Fungicide application appeared to be more but not totally effective as compared with other pesticides although Vydate and V+S treatments influenced some yield components more than fungicide treatment eventhough pure seed yield differences were not significant (Table 8). Little information on the effect of specific plant pathogens on cocksfoot seed yield exists although Latch (1980), and Labruyere (1980) have all reported that stem rust (*Puccinia graminis*) and stripe rust (*P. reformis*) can reduce seed yield. More recently, Welty (1991), and Bouchet, (1987) have reported significant yield increases following the control of eye spot (*M. rubricosum*). Rolston *et al* (1989) has also found that control of eye spot can increase seed yield in cocksfoot in New Zealand. The seed yield response to fungicide application in the apparent absence of fungal disease has also been recorded in cocksfoot (Rolston *et al*, 1989), perennial ryegrass (Hampton and Hebblethwaite, 1984; Hampton, 1986; Horeman, 1989) and prairie grass (Rolston *et al*, 1989). When seed yield components were recorded, the major response was an increase in seeds per spikelet, or more accurately, better floret site utilization

because of reduction in seed abortion (Hampton, 1986). While fungicide application can result in delayed leaf senescence, thus presumably allowing extended photosynthetic activity through extending green leaf duration and more assimilate to support seeds, the reasons why this response occurs is still not clear. Marshall (1985) found that fungicide treatment maintained the photosynthetic activity of tillers and resulted in an improved supply of assimilates to developing perennial ryegrass seeds at a time when they are normally prone to abort, thereby improving floret site utilization. Hampton (1986) has also suggested that control of the micro-organisms involved in leaf senescence may be an explanation, or that growth regulatory properties of the fungicides (eg. hormonal changes) may be involved, but this is still to be confirmed. Delayed senescence of leaf tissue after fungicide application has also been reported in cereals (e.g. Priestley 1981; Priestley and Bayles 1982). It was reported that fungicides of the benzimidazole group in particular, may have a direct biochemical effect on senescence by retarding chlorophyll breakdown (Staskawicz *et al.*, 1978). Another possibility could be failure to recognise a pathogen or pathogens associated with leaf scorching and premature senescence of cocksfoot which are being controlled by fungicide application. This situation has recently been reported for *Didymella* spp. in New Zealand cereals (Cromeey *et al.*, 1994).

The possible improvement in seed yield by Vydate and V+S treatment (Table 8) as well as significantly higher numbers of florets (Table 9) may be due to improved plant growth following Vydate treatment as reported by Spaul and Mewton (1984), Clements *et al.* (1985) and Smolik (1977) presumably by reducing or inactivating nematode activity (Bezooijen, 1984). Vydate (oxamyl) is systemic in action and has mainly a upward movement from foliar treatment (Anon, 1993) which provides protection to the inflorescences from attack by nematodes. However the significant increase in numbers of floret in Vydate and V+S treated plots (Table 9) did not produce significantly higher seed yield compared to fungicide treatment suggesting a better or positive effect on floret site utilization following the use of fungicide.

Disease incidence

Attempts to verify Koch's postulates with an authenticated culture of *C. rathayi*, with the isolated bacterium from the previous year's crop or with isolated freeliving nematodes obtained from the field all failed to produce any symptoms of the Rathay's Disease. Although this demonstrates the inability of field isolates of nematodes and bacteria to produce the expected disease symptoms it does not explain why the inoculation of an authenticated culture of *C. rathayi* originally isolated from cocksfoot was unsuccessful. Perhaps lack of maintenance of high relative humidity following inoculation, or high green house temperatures may have been a limiting factor in this Koch's postulate study. Despite this, growing the suspected field isolated bacterium on different selective media (Appendices 17 a,b and c) and unsuccessful growth on diagnostic TTC medium (Appendix 17d) indicates that the consistently associated bacterium from this seed crop was likely to be saprophytic. Failure to establish symptoms of Rathay's Disease in cocksfoot has previously been documented by Sabet (1954) with the nematode *Anguina* spp. and others (Dowson & D'Oliviera 1935) even though they suspected that some unidentified nematode species may be involved in transmitting this bacterium. Goodey, (1940) previously suggested that four species of nematodes may play a important role in the etiology of Rathay's Disease. Smolik (1977) and Stanton *et al.* (1981), suggested that nematodes are the most important consumers in grassland and plant feeding nematodes may constitute about 70% of the nematode fauna in grass fields. Little information is available on Rathay's Disease in New Zealand since its recorded appearance is apparently rare and sporadic in New Zealand (eg. Johnstone, 1956). The nonsignificant pure seed yield in fungicide, Vydate and V+S treatments suggests that nematodes and bacteria were not a problem in cocksfoot seed production at this site and in this climate. It seems more likely that timely and specific management practices, seed shattering and climatic variation may possibly be implicated in affecting yield.

Effect of fungicide on seed yield and yield components (Trial 2).

A comparison of the effect of three different fungicides on seed production in the cocksfoot cultivar Tekapo revealed that none were effective in consistently increasing seed yield and yield components (Table 15). In terms of controlling foliar disease,

however, the fungicide Alto (cyproconazole) appeared to be more effective (Table 16). Although Alto reduced disease severity significantly compared to the control, seed yield was not increased significantly (Table 15) possibly because of the age of the crop stand. Why fungicide failed to improve seed yield in this trial with this cultivar (Tekapo) compared to the positive results obtained by Wilson (1995) at the same site is surprising. A variety of reasons could be associated with this result including types of fungicide, cultivar etc. In most fungicidal trials significant yield increases have been reported in different grasses and in cocksfoot even in the absence of pathogens as discussed in the previous section. However, in other grasses like tall fescue Welty (1989b) showed no increase in seed yield due to fungicide treatment. Similarly in lodged perennial ryegrass Burbidge (1977) did not obtain seed yield responses through the application of tridemorph or carbendazim. Welty (1990) in work in Oregon observed seed yield increases in one cultivar of perennial ryegrass but not in another when propiconazol was applied twice during ear emergence. The lack of effects on seed yield (Table 15) in the present trial may be associated with cultivar, as recognized in Welty's (1990) trial with ryegrass. Also, fertile tiller numbers in cv Tekapo in general was relatively high compared (Table 15) to the other four cultivars considered in trial 1 (Table 3). In Wilson's trial (1995) she used terboconazole, a systemic fungicide recommended for use on grasses. In the present trial, other fungicides were selected to provide chemicals with different modes of action. Alto has a systemic and curative action while both Bravo and Bavistin are protectant chemicals. Finally, since seed yield was obtained from a three year old crop stand, considerable variation existed in plant populations within individual plots. The high cv% in most characters may, as a result, have contributed to nonsignificant seed yield results.

Seed quality

Most seed quality characters studied were unaffected by fungicide application. However, germination was significantly affected by fungicide treatment. In single floret germination tests only Bavistin significantly improved germination (Table 18), although Alto was found to be effective in reducing disease severity. In multiple floret germination tests again Bavistin and Bravo improved germination significantly.

The effectiveness of Bavistin in improving seed yield has been previously investigated by several investigators (Hampton and Hebblethwaite 1984 and 1985, Hampton, 1986) in perennial ryegrass. A similar response did not occur in this trial, although it must be recognised that stand age and possibly differences in cultivars susceptibility to fungicides as recognised by Welty (1990) may be limiting factors.

CONCLUSION

Four cocksfoot cultivars (two from New Zealand and two from Japan) were evaluated in a three year old stand for their seed production capabilities and to explore the possibilities of using a nematicide, bactericide alone or in combination to both improve seed yield compared to more traditional fungicidal application in cocksfoot seed crops. The New Zealand cultivar Grasslands Wana outyielded the remaining 3 cultivars while New Zealand cultivar Grasslands Kara and the Japanese cultivar Makibamidori performed equally in terms of seed yield. The remaining Japanese cultivar Akimidori performed poorly and yielded about 71%, 33% and 30% lower seed yield than cvs Wana, Kara and Makibamidori respectively.

The higher seed yield in cv Wana was largely due to its higher number of fertile tillers/m² at anthesis as well as higher numbers of florets/head. Similar yields in cv Kara and Makibamidori were associated with heavier seed weight. The number of spikelets per seed head was also higher in cv Wana. Despite similar yields of harvested seed and significantly higher numbers of florets produced by the New Zealand cultivar Kara, it failed to outyield cv Makibamidori because of the production of more empty florets, presumably due to higher levels of abortion.

The percentage recovery of pure seed following cleaning was lowest in cv Akimidori compared to the remaining cultivars. In fact the percent purity in different seed fractions as well as seed yield from each respective seed cleaning fraction was also lowest in cv Akimidori. Large amounts of pure seed were lost during seed cleaning, with the highest percentage of pure seed in cv Wana occurring in the screen and blow fractions, mainly due to lower seed weight.

The marked decline in seed yield from this third year crop compared to the second year crop studied at the same site by Wilson (1995) may be caused by larger numbers of florets which failed to fill. In this trial, a number of things could have occurred which resulted in reduced seed recovery or floret site utilization. One possibility is the increased numbers of tillers placed increased pressure on assimilate supply, thus leaving

less for the developing panicle. This would have resulted in seeds not receiving enough assimilate to develop, and consequently they aborted. Another possibility, is that the stems and leaves did not remain green with a subsequent restriction on photosynthetic duration. Another possibility is that environmental condition during anthesis were not favourable. During November 1994 (peak anthesis) there was very high rainfall (183mm compared to only 69 mm in the 20 year average) (Appendix 5). Hill (1980) stated that grass florets generally do not open unless the weather is warm and dry, and that pollen is rapidly washed out of the air by rain. Also the duration of anthesis can be significantly prolonged during wet weather. This could mean that in the case of this 3rd year crop, a lot of florets were not successfully pollinated due to rain, thus reducing floret. Such a situation may also have resulted in uneven ripening of the seed crop since uneven ripening is a major contributing factor to poor floret site utilization Meiger (1985).

Despite this unfavourable weather conditions during seed development stage still enabled cv Wana to produce higher amounts of seed. Although cv Kara had similar numbers of florets and higher seed weight, suggesting it also has similar seeding potential to cv Wana proper site utilisation occurred. Of the two Japanese cultivars examined in this trial cv Makibamidori performed significantly better than cv Akimidori. Since cv Akimidori develops earlier than the New Zealand cultivars and has a lower wintering ability as indicated Jiro (1989) this combined with weather conditions during anthesis and early seed development led to production of more empty florets eventhough cv Akimidori has same yield potential as cv Makibamidori. Possibly these two cultivars may produce more seed if sown in Canterbury where the climate is better suited to herbage seed production.

Similar pure seed yields in fungicide, Vydate and V+S treatments suggested that nematodes and bacteria were not a problem for seed production in this study. Failure to produce symptoms of Rathay's disease when disease free plants were artificially inoculated with authenticated culture or with nematodes obtained from the fields suggest that neither were capable of causing the symptoms of the disease. Inability of field isolated bacterial culture to grow on selective and on diagnostic media indicates that the bacteria consistently associated with this crop is not *C. rathayi*.

Still fungicide application especially Folicur appear equally effective as nematicide or combination of nematicide and bactericide in cocksfoot seed production compared to bactericide application. As fungicide application was shown to be cost effective way of increasing seed yield and a single application at ear emergence provided the most cost effective return, so it can be used as a essential management tool for cocksfoot seed crop along with other management practices.

Work that needs to be looked at more closely is the ability of cocksfoot cultivars, particularly cv Wana, to utilise stem reserves as it may have been a factor in this trial. Roy and Rolston (1994) have suggested that in ryegrass, 14-51% of stored stem reserves are utilized by the seed head, but similar data are not available in cocksfoot as a species, let alone in individual cultivars.

In cocksfoot, breeding had attempted to improve seed retention on the plant, thus reducing shattering losses (Falcinelli *et al*, 1994). The duration of anthesis is another area that could be examined in considering long term seed yield improvement in cocksfoot. Cocksfoot appears to have a long period of anthesis, and subsequently a long period for seed maturity. As Meijer (1985) found in perennial ryegrass, uneven ripening of the seed crop contributes to poor floret site utilization. A reduction in this flowering time could be carried out through site selection (climate) or maybe through breeding programmes.

Work is still required to examine the effect of fungicides on seed production in the absence of disease. Is seed yield improved by delaying the senescence of photosynthetic tissue (Hampton and Hebblethwaite 1984), or by applied fungicide exerting a cytokinin-like action on the seed head making it a stronger sink for assimilates (Marshall 1985)? Is a combination of both of these things important or is there another factor involved? This requires further investigation.

In the second trial from the same crop stand with cv Tekapo, three 3 different groups of fungicides were tested to find their efficacy in improving seed yield. Although the application of Alto (cyproconazole) provided good control of eye spot it failed, in

contradiction to most other findings, to improve seed yield. Differences in the types of fungicide or/cultivar might be responsible for this since Welty (1990) found no increase in seed yield due to fungicide application in perennial ryegrass. Fungicide application was also found not to alter seed quality although germination results in single and in multiple florets did vary significantly. Nevertheless in all cases seed germination percentage was well above the commercially accepted level (>85%) for cocksfoot. Fungicide application might have been expected to increase TSW (Latch, 1980) but in this trial this did not occur. This area also requires further investigation.

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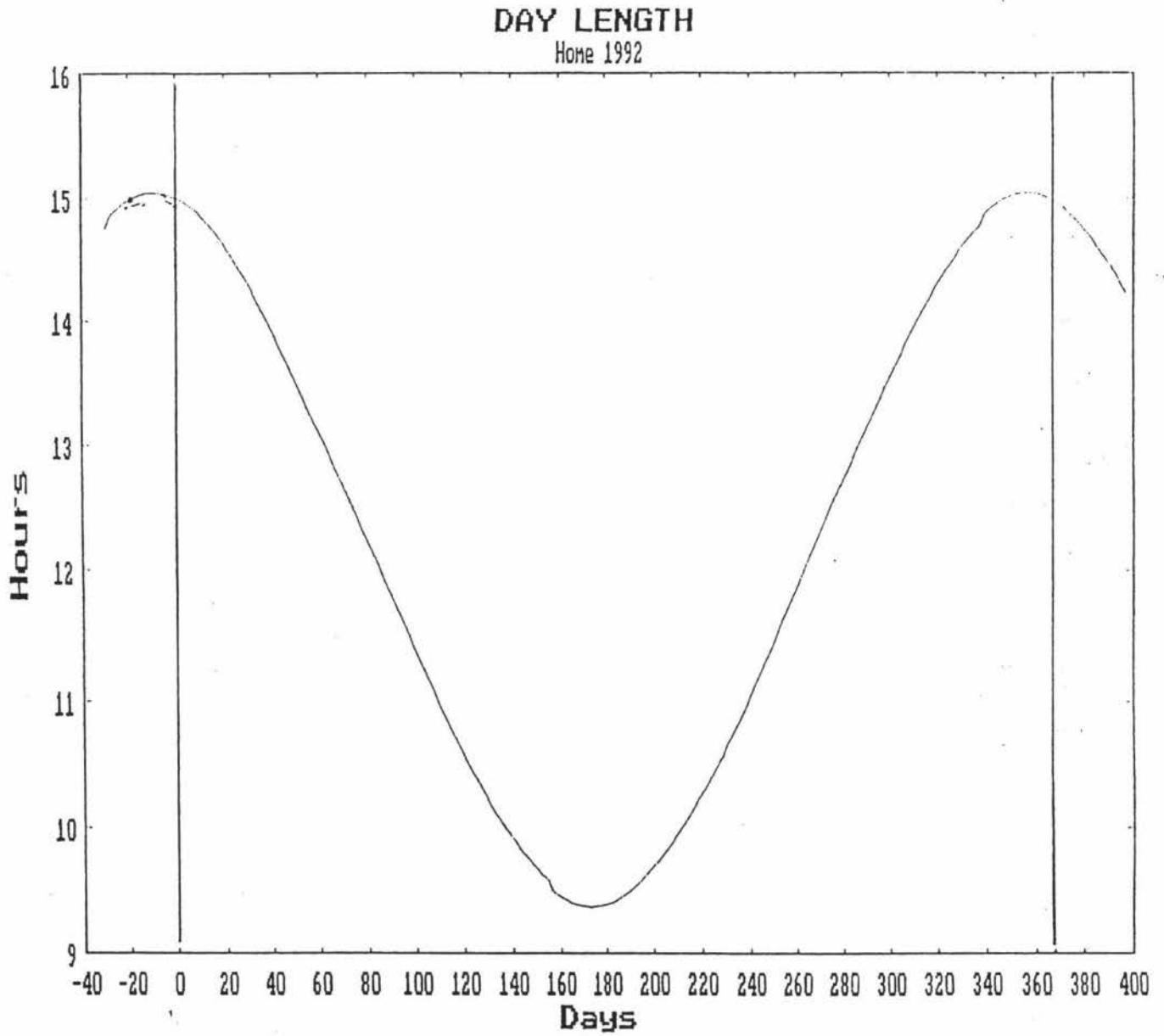
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APPENDICES



Appendix 1. Yearly daylength pattern in Palmerston North

Appendix 2. Metrological data 1994 and January, 1995 at Aorangi Lowland Research Station

		Aug	Sept	Oct	Nov	Dec	Jan
Rainfall	Maximum daily	3.8	6.1	5.0	11.4	6.4	6.4
	Total monthly	75.0	139.6	75.2	183.0	38.6	51.4
	20 years average	79.7	78.9	78.9	66.9	78.4	63.4
Max. Air Temp.	Maximum	15.4	16.7	20.9	22.4	24.4	27.2
	Minimum	9.1	9.2	10.7	11.8	16.1	17.2
	Mean	13.1	13.7	15.6	17.0	20.2	22.4
	20 year average	13.5	15.1	15.1	18.7	20.8	22.2
Min Air Temp.	Maximum	9.7	10.0	11.8	14.1	15.9	17.4
	Minimum	-2.1	-1.9	-1.5	2.9	4.4	2.0
	Mean	4.5	5.3	6.6	9.9	10.1	11.1
	20 year average	4.5	6.7	6.7	9.5	11.2	12.3
Min Grass temp	Maximum	9.5	10.2	11.9	13.5	16.0	17.0
	Minimum	-6.5	-6.1	-5.3	-1.5	0.0	-2.6
	Mean	2.0	1.9	4.0	7.3	7.6	8.2
Radiation	Maximum	18.7	22.1	32.5	37.4	40.6	39.4
	Minimum	4.1	7.0	7.0	6.3	10.7	14.1
	Mean	11.9	15.4	22.4	25.0	29.9	31.3

Appendix 3. Minimum Grass temperature for the months of October and November, 1994 at Aorangi.

Days	October °C	November °C
1	-3.2	-1.5
2	4.0	1.9
3	-5.3	3.9
4	-1.8	4.4
5	6.8	2.6
6	1.3	9.4
7	8.8	12.5
8	2.6	8.1
9	4.6	7.1
10	8.2	9.7
11	3.4	7.0
12	8.0	8.1
13	-0.1	13.0
14	6.6	13.5
15	-4.5	13.1
16	-1.4	4.6
17	7.0	9.7
18	8.3	6.3
19	4.3	2.5
20	6.1	8.5
21	6.7	12.8
22	-0.9	9.1
23	-0.6	9.8
24	0.5	3.8
25	4.7	4.5
26	6.9	5.8
27	7.0	-0.1
28	5.4	11.7
29	11.9	10.2
30	9.9	6.8
31	8.9	-

Appendix 4. Nematode incidence and foliar disease incidence (eye spot) in 4 cocksfoot cultivars at harvest.

Variety	Nematode Population/4gm leaf tissue		Disease incidence		
	Before treatment	At harvest	Vegetative growth stage	Peak Anthesis	At Maturity
Wana	22.94	28.19	1	1	1.06
Kara	25.81	27.81	1	1	1.19
Makibamidori	25.69	29.69	1	1	1.06
Akimidori	24.94	28.75	1	1	1.06
LSD (0.05)	NS	NS	NS	NS	(NS)

**** Means are Average of 16 observations**

Appendix 5. Effect of 4 pesticide and 4 cultivar treatment combinations on seed yield and TSW (at 10% SMC).

Treatment combination	Harvested Yield (g)/m²	Yield/ plot (g)/ m²	% Pure seed Recovered	Thousand seed wt. (g)
Wana + Fungicide	150.56	103.33	68.63	0.091
Wana + Vydate	115.94	86.21	74.36	0.095
Wana + Streptomycin	80.46	56.07	69.69	0.098
Wana +(V+S)	107.04	74.21	69.33	0.095
Kara + Fungicide	75.46	55.10	73.02	0.114
Kara + Vydate	93.91	67.75	72.14	0.113
Kara + Streptomycin	74.24	53.85	72.54	0.108
Kara + (V+S)	87.08	64.08	73.59	0.112
Makibamidori + Fungicide	54.74	37.86	69.16	0.114
Makibamidori + Vydate	95.39	67.77	71.05	0.111
Makibamidori + Streptomycin	52.13	33.83	64.90	0.111
Makibamidori + (V+S)	116.86	76.01	65.04	0.107
Akimidori + Fungicide	56.59	21.92	38.73	0.106
Akimidori + Vydate	42.94	20.04	46.67	0.101
Akimidori + Streptomycin	46.95	21.75	46.33	0.111
Akimidori + (V+S)	74.59	29.66	39.76	0.101
LSD 0.05	36.91	25.78	8.79	0.008
CV%	31.36	33.38	9.79	5.27

Appendix 6. Interaction between cultivar, pesticide and cultivar X pesticide on seed yield.

Character	Sources of variance	DF	F Value	Significance
Harvested yield/m ²	Cultivar	3	13.52	**
	Pesticides	3	4.59	**
	Cultivar X Pesticides	9	2.68	*
Pure seed Yield/m ²	Cultivar	3	26.76	**
	Pesticides	3	4.04	*
	Cultivar X Pesticides	9	2.33	*

** Significant at 0.01% level

* Significant at 0.05% level

Appendix 7. Effect of 4 pesticides and 4 cocksfoot cultivars combinations on seed yield components.

Treatment combination	No. of fertile tiller m²	No of florets/ spikelets	No of florets/ Head	No. of spikelets/ head
Wana + Fungicide	827	4.40	1825.04	415.15
Wana + Vydate	695	4.51	1923.89	426.10
Wana + Streptomycin	530	4.60	1899.64	414.30
Wana + (V+S)	676	4.52	1732.59	386.70
Kara + Fungicide	547	4.91	1565.73	320.10
Kara + Vydate	635	4.66	1856.39	399.80
Kara + Streptomycin	480	4.73	1598.66	337.40
Kara + (V+S)	560	4.55	1704.96	376.55
Makibamidori + Fungicide	480	4.65	1237.63	266.85
Makibamidori + Vydate	603	4.38	1476.27	336.80
Makibamidori + Streptomycin	475	4.66	1582.11	339.70
Makibamidori + (V+S)	707	4.60	1659.59	356.60
Akimidori + Fungicide	545	4.48	1261.54	282.30
Akimidori + Vydate	645	4.87	1445.62	298.30
Akimidori + Streptomycin	590	4.50	1306.80	290.40
Akimidori + V+S	475	4.62	1638.70	354.30
LSD 0.05	170.35	0.35	419.23	93.66
CV%	20.25	5.39	18.57	19.14

Appendix 8. Interaction between cultivar, pesticide and cultivar X pesticide on different seed yield components

Character	Sources of variance	DF	F Value	Significance
No. of spikelets/head	Cultivar	3	9.16	**
	Pesticides	3	2.08	NS
	Cultivar X Pesticides	9	0.85	NS
No. of florets/spikelets	Cultivar	3	1.92	NS
	Pesticides	3	0.11	NS
	Cultivar X Pesticides	9	1.66	NS
No. of florets/head	Cultivar	3	8.24	**
	Pesticides	3	2.09	NS
	Cultivar X Pesticides	9	0.88	NS
Thousand seed weight(TSW)	Cultivar	3	32.80	**
	Pesticides	3	0.94	NS
	Cultivar X Pesticides	9	1.63	NS
Fertile tiller/m ²	Cultivar	3	4.04	*
	Pesticides	3	3.10	*
	Cultivar X Pesticides	9	2.34	*

** Significant at 0.01% level

* Significant at 0.05% level

Appendix 9. Effect of 4 pesticide and 4 cocksfoot cultivar treatment combinations on pure seed recovered from different fractions and nematode populations before treatment.

Treatment combination	Pure seed yield from machine clean material	Pure seed yield from screen leftover	Pure seed yield from blow leftover	Nematode population before treatment
Wana + Fungicide	32.68	20.94	49.72	20.25
Wana + Vydate	24.53	22.39	39.31	22.25
Wana + Streptomycin	15.55	12.01	28.52	24.25
Wana + (V+S)	21.01	8.14	44.85	25.00
Kara + Fungicide	14.25	12.05	28.81	25.25
Kara + Vydate	29.68	15.18	22.89	24.25
Kara + Streptomycin	26.63	20.12	9.79	27.00
Kara + (V+S)	29.15	12.64	23.55	26.75
Makibamidori + Fungicide	19.41	12.09	6.36	24.50
Makibamidori + Vydate	30.93	19.04	17.80	23.50
Makibamidori + Streptomycin	24.12	5.67	4.05	25.50
Makibamidori + (V+S)	51.37	20.88	3.76	29.25
Akimidori + Fungicide	13.55	6.09	2.28	21.50
Akimidori + Vydate	13.22	4.99	1.84	25.75
Akimidori + Streptomycin	9.57	7.29	4.89	23.25
Akimidori + (V+S)	13.78	7.02	11.29	29.25
LSD 0.05	13.35	9.99	66.60	NS
CV%	40.59	54.33	69.49	37.83

Appendix 10. Interaction between cultivar, pesticide and cultivar X pesticide on seed cleaning processes.

Character	Sources of variance	DF	F value	Significance
Pure seed yield from clean fraction	Cultivar	3	11.23	**
	Pesticides	3	3.76	*
	Cultivar X Pesticides	9	3.40	**
Pure seed yield from screen fraction	Cultivar	3	6.33	**
	Pesticides	3	1.02	NS
	Cultivar X Pesticides	9	2.66	*
Pure seed yield from blow fraction	Cultivar	3	24.76	**
	Pesticides	3	2.04	NS
	Cultivar X Pesticides	9	0.99	NS

** Significant at 0.01% level

* Significant at 0.05% level

Appendix 11. Effect of 4 pesticide and 4 cocksfoot cultivar treatment combinations on seed quality, nematode population at harvest and foliar disease incidence (1-5) at harvest.

Treatment combination	Germination (single floret)	Germination (multiple florets)	Nematode population at harvest	Disease incidence at harvest
Wana + Fungicide	88.50	82.38	37.75	1.00
Wana + Vydate	90.38	72.25	17.25	1.25
Wana + Streptomycin	89.13	80.25	39.50	1.00
Wana + (V+S)	92.25	85.13	18.75	1.00
Kara + Fungicide	91.50	85.75	33.25	1.25
Kara + Vydate	91.13	88.50	19.00	1.50
Kara + Streptomycin	91.00	84.13	41.25	1.00
Kara + (V+S)	91.63	91.00	17.75	1.00
Makibamidori + Fungicide	87.63	87.63	40.00	1.00
Makibamidori + Vydate	93.38	90.88	19.50	1.25
Makibamidori + Streptomycin	91.63	87.88	37.25	1.00
Makibamidori + (V+S)	92.25	89.50	22.00	1.00
Akimidori + Fungicide	92.63	93.25	37.25	1.25
Akimidori + Vydate	95.00	94.88	19.75	1.00
Akimidori + Streptomycin	93.88	93.63	39.25	1.00
Akimidori + (V+S)	94.13	94.75	18.75	1.00
LSD 0.05	3.63	6.16	10.06	0.41
CV%	2.77	4.93	24.73	26.17

Appendix 12. Interaction between individual treatments, cultivars, pesticides and cultivar X pesticide on foliar diseases and nematode population.

Character	Sources of variance	DF	F value	Significance
Foliar disease at harvest	Cultivar	3	0.74	NS
	Pesticides	3	2.70	NS
	Cultivar X Pesticides	9	0.74	NS
Nematode population at harvest	Cultivar	3	0.21	NS
	Pesticides	3	38.90	**
	Cultivar X Pesticides	9	0.33	NS
Nematode population before any treatment	Cultivar	3	0.32	NS
	Pesticides	3	0.73	NS
	Cultivar X Pesticides	9	0.12	NS

** Significant at 0.01% level

* Significant at 0.05% level

Appendix 13. Effect of 4 pesticide and 4 cocksfoot cultivar treatment combinations on seed quality.

Treatment combination	% purity in machine clean seed	% purity in screen fractions	% purity in blow fractions	% Multiple seed in pure seed
Wana + Fungicide	76.21	66.23	65.14	21.95
Wana + Vydate	76.16	71.62	73.50	21.75
Wana + Streptomycin	73.85	59.55	67.84	24.52
Wana + (V+S)	69.95	56.75	67.93	27.70
Kara + Fungicide	63.71	67.43	71.54	11.72
Kara + Vydate	78.58	75.29	55.66	19.48
Kara + Streptomycin	79.62	74.57	58.73	18.25
Kara + (V+S)	79.17	70.37	63.08	18.78
Makibamidori + Fungicide	74.57	68.13	50.54	23.19
Makibamidori + Vydate	75.80	75.92	53.93	19.59
Makibamidori + Streptomycin	72.61	64.55	32.27	21.81
Makibamidori + (V+S)	75.76	73.33	17.57	18.53
Akimidori + Fungicide	55.94	47.58	11.61	26.45
Akimidori + Vydate	59.52	47.46	16.95	27.02
Akimidori + Streptomycin	61.81	48.05	29.17	28.41
Akimidori + (V+S)	55.83	45.40	21.74	30.53
LSD 0.05	18.17	9.88	19.72	9.86
CV%	18.11	10.99	29.26	30.78

Appendix 14. Interaction between cultivars, pesticides and cultivar X pesticide on seed quality.

Character	Sources of variance	DF	F value	Significance
% purity in clean fraction	Cultivar	3	6.60	**
	Pesticides	3	0.48	NS
	Cultivar X Pesticides	9	0.48	NS
% purity in screen fraction	Cultivar	3	42.76	**
	Pesticides	3	2.78	NS
	Cultivar X Pesticides	9	1.45	NS
% purity in blow fraction	Cultivar	3	41.90	**
	Pesticides	3	0.99	NS
	Cultivar X Pesticides	9	2.47	*
Germination (single floret)	Cultivar	3	6.48	**
	Pesticides	3	3.34	*
	Cultivar X Pesticides	9	0.94	NS
Germination (multiple floret)	Cultivar	3	29.21	**
	Pesticides	3	2.45	NS
	Cultivar X Pesticides	9	2.24	*
Proportion of multiple floret unit	Cultivar	3	7.36	**
	Pesticides	3	0.62	NS
	Cultivar X Pesticides	9	0.58	NS

** Significant at 0.01% level

* Significant at 0.05% level

Appendix 15. Nematode genera isolated from cocksfoot leaf:

- 1) *Panagrolaimus*
- 2) *Diplogasrer*
- 3) *Cephalobus*
- 4) *Rhabditis*

Appendix 16. Nematode genera isolated from cocksfoot field (soil) :

- 1) *Clarkus*
- 2) *Pratylenchus*
- 3) *Helicotylenchus*
- 4) *Tylenchus*
- 5) *Panagrolaimus*
- 6) *Cephalobus*
- 7) *Plectus*

Appendix 17. Media used for isolation and culture of bacteria**a) Semi selective medium for detecting *C. michiganensis***

Pourite - stock solution (1drop/ml)...	250 U _i
K ₂ HPO ₄ . 3H ₂ O....	2.62 g
KH ₂ PO ₄	0.50 g
MgSO ₄ . 7H ₂ O	0.25 g
Boric Acid	1.50 g
Sucrose	10.0 g
Yeast Extract	0.10 g
Agar	12.0 g
Distilled Water	980 ml

After dissolving the above in the distilled water, autoclaved at 120 C at least at 15 psi for 15 minutes and cool in water bath to 45-50 C

Following cooling add:

Nicotinic Acid	100 mg dissolved in 20 ml sterile distilled water
Nalidixic Acid (Sodium Salt)...	30 mg dissolved in 1 ml of 0.1 M NaOH
Cyclohexamide.....	200 mg dissolved in 1 ml absolute methanol.

b) Nutrient Glucose Agar (NGA)

	<u>gm.</u>
Beef Extract (Difco)	3.00
Peptone	5.00
Glucose	2.50
Agar	15.00

c) Nutrient Broth Yeast Extract Agar (NBY)

	<u>gm.</u>
Nutrient broth (Difco)	8.00
Yeast extract	2.00
K ₂ HPO ₄	2.00
KHPO ₄	0.50
Agar	15.00

Add after Autoclaving separately, 50 ml 10% glucose 1.0 ml MgSO₄, 7H₂O

d) TTC Diagnostic Medium

	<u>gm.</u>
Glucose	10.00
Peptone	10.00
Casmino acid (Casein hydrolysate)	01.00
Agar	18.00