THE BEHAVIOURAL BIOLOGY OF ARGENTINE STEM WEEVIL IN RELATION TO HOST-PLANT CHARACTERS.

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STEPHEN PILKINGTON

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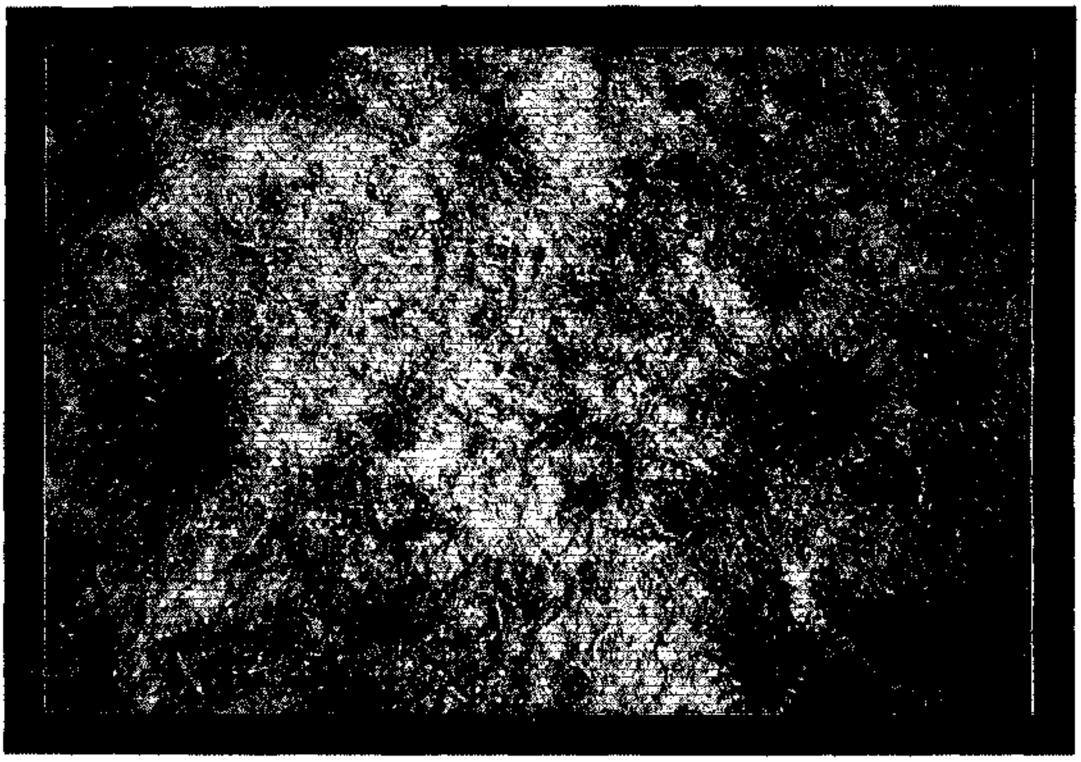
FRONTISPIECE



Severe Pasture Damage by Argentine Stem Weevil.

(Photo by D.L. Gaynor)

ABSTRACT

Literature on the biology, damage, and control of Argentine stem weevil (ASW), *Listronotus bonariensis* (Kuschel), is reviewed. Plant resistance with respect to control in other insects is reviewed in detail.

Orientation, feeding and oviposition behaviour of ASW were investigated in relation to ryegrass.

The visual and olfactory components of orientation were examined. Weevils did not discriminate between coloured nutrient agar plugs, nor between treatments in an olfactometer. Antennectomised weevils could discern between tissue from resistant ryegrass (infected with *Acremonium lolii* endophyte) and susceptible ryegrass (endophyte-free) when feeding, but not in the olfactometer. Host selection is probably mediated by a touch/taste system. The "apparency" of ryegrasses offers the most likely mechanism for location by ASW.

ASW shows a gradation of feeding preferences between leaf tissue from twelve ryegrass cultivars. Annual cultivars were most preferred, then hybrids, perennials without endophyte, and lastly, perennials containing endophyte. In feeding trials with clonally replicated plants, weevils strongly preferred endophyte-free tissue. When restricted to endophyte-infected tissue, ASW feeds towards the leaf tip, where the lowest concentration of endophyte occurs. The amount of food consumed was inversely proportional to endophyte concentration. Weevils also prefer to feed on annual cultivars which have the least fibre and lowest tensile strength. Partial isolation of an extract that prevents ASW feeding, obtained from endophyte-infected ryegrass tissue after partitioning and dialysis, revealed a compound similar to "peramine". Age, size, and sex of weevils made no difference to the amount of food consumed.

Oviposition preferences paralleled feeding preferences in twelve ryegrass cultivars. Plant characteristics examined in relation to oviposition were endophyte concentration and distribution, age, fibre content, and tiller diameter. Endophyte concentration and fibre content in ryegrass sheath material followed a similar pattern, low in annuals, intermediate in hybrids and endophyte-free perennials, and high in endophyte-infected perennials. Tiller age had no effect on oviposition preference. Tiller diameter was positively correlated with oviposition preference, large diameter tillers (annual cultivars) being preferred. The behavioural components of oviposition are described, and evidence found which suggests a possible role for epideictic pheromones.

The external receptors probably used in host-plant selection were identified and described. The antennae, mouthparts, tarsi, and ovipositor possess numerous sensilla, any of which could respond in the host selection process.

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CHAPTER 1

INTRODUCTION

"The susceptibility or resistance of different plant species or varieties to an insect species varies with their suitability or unsuitability, respectively, for the establishment of an insect population" (Saxena 1985). Other things being equal, the greater the suitability of a plant, the greater its susceptibility and the greater the potential for resultant damage. Therefore, in order to understand the role of behavioural responses in the susceptibility or resistance of plants to an insect species, it is necessary to compare the responses of the insect to known susceptible and resistant plants, and, to examine the role of plant characters in determining such responses.

There are three main types of behavioural response by an insect which determine selection or rejection of a plant. These collectively form the host-plant selection process. These are: 1 - orientational responses, determining the insect's arrival on or avoidance of a plant in response to attractant, neutral, or repellent stimuli; 2 - feeding responses, determining the quantity of food ingested from the plant, and 3 - oviposition responses.

The various plant characters which may influence these behavioural responses of insects act in two ways: 1 - by providing sensory stimuli, and 2 - by providing mechanical features which may facilitate or hamper behaviour. The sensory stimuli from various plant characters may be perceived at a distance or by contact. The distance-perceivable characters may involve visual (colour, form, shape), hygro (water vapour), and olfactory (non-aqueous volatiles) stimuli. The contact-perceivable characters are chemical (gustatory) and physical (tactile) stimuli.

In order to understand the host-plant selection process of an insect and thus mechanisms of plant resistance and the characters involved, a comparison of these three behavioural responses to a range of resistant and susceptible plant species or varieties is essential. Then, by manipulating either the insect's behavioural responses, or, more effectively the plants characteristics, new control strategies may be developed against the insect.

The objective of the present study is to provide fundamental information which could lead to future population management of the Argentine stem weevil, *Listronotus bonariensis* Kuschel, (Coleoptera : Curculionidae). This insect, referred to as ASW throughout the text, is a major pest of pasture grasses and graminaceous crops throughout New Zealand. It has been estimated to cause losses in excess of $150 million per annum (Pottinger pers. comm. 1985), and adult populations in excess of 20 million per hectare are common (Pottinger 1977). Considering New Zealand's reliance on grassland agriculture, such losses, if sustained, could be exceptionally detrimental to the national economy. Both adults and larvae feed on graminaceous plants, with the larval stage considered the most destructive due to its stem-mining habit and hence difficulty of control. Larval attack is more evident in dry summers and on short-rotation ryegrasses, where it can result in up to 98% tiller mortality (Kelsey 1958). However, as larval stem weevils are restricted to their host plant for their entire development, the amount of damage is ultimately determined by the discriminatory behaviour of the ovipositing female in locating and selecting suitable host plants, hence only the adult stage is considered in this thesis.

Of the graminaceous plants which are considered hosts of ASW, ryegrasses (*Lolium* spp.) are preferred (Prestidge *et al.* 1985c). Early research on ryegrasses recognised their variable susceptibility to ASW (Kelsey 1958; Timlin 1964; Pottinger 1961a, b, and 1977; Barker *et al.* 1981; and Prestidge *et al.* 1982). Many short rotation ryegrass cultivars are highly susceptible, while many perennial cultivars exhibit variable resistance (Goldson 1982; Kain *et al.* 1982a and b; Gaynor and Hunt 1983; and Barker *et al.* 1984). This resistance has been linked to the presence of an endophytic fungal infection within certain ryegrass plants (Prestidge *et al.* 1982; Mortimer *et al.* 1982), although Gaynor and Hunt (1983) noted genotypic differences unrelated to endophyte content. It is because of such apparent variability that a selection of available ryegrass cultivars was examined for a), their susceptibility and resistance to adult ASW with respect to the three behavioural components of orientation, feeding, and oviposition, and b), the plant characters which influenced these responses.

CHAPTER 2

LITERATURE REVIEW.

This chapter is intended both to comprehensively review relevant publications on ASW, and to provide selective examples of many plant resistant mechanisms which affect the three behavioural components of host-plant selection. The latter encompasses a vast amount of literature, consequently most examples are referred to in detail.

The Study Insect.

Argentine stem weevil (ASW), *Listronotus bonariensis*, was first described by Hustache (1929) from a locality near Buenos Aires, Argentina. Kuschel (1955; 1972) reported that this insect is of South American origin, being widespread in Argentina, Uruguay, Chile and Bolivia. Most literature pertaining to this pest is, however, of New Zealand origin, where the weevil was first collected in 1927 (Marshall 1937). Kuschel (1972), however, believed the insect was already common and causing appreciable damage to pastures by this time. Subsequent work has increasingly shown the importance of ASW in graminaceous crops (Table 1).

Biology.

Oviposition behaviour of adult female ASW on cereals and grasses was reported by Morrison (1938), Blair and Morrison (1949), Kelsey (1958), and Pottinger (1961a). The female chews a hole in the outer epidermis of the host plant, then deposits between 1 and 6 eggs (average 1.9), approximately 1 mm below the insertion hole (Blair and Morrison 1949; Pottinger 1961a). Each female lays between 11 and 62 eggs (average 27), during the entire oviposition period of between 8 and 26 days (average 18) (Ahmad 1978). The position of egg deposition is variable, but is generally within 50 mm of the ground in the vicinity of the ligule (Kelsey 1958; Pottinger 1961a) and only on stems of at least 1 mm in diameter (Pottinger 1961a).

Table 1.

Host Plants of Argentine Stem Weevil.

Plant Species.	Scientific Name.	Author.	Date.
* Wheat	*Triticum aestivum*	Morrison	(1935).
* Barley	*Hordeum sativum*	"	"
* Ryegrass (perennial)	*Lolium perenne*	"	"
* " (Italian)	" *multiflorum*	"	"
* " (hybrid)	" *hybridum* vars.	"	"
* Danthonia	*Danthonia pilosa*	"	(1938).
* Cocksfoot	*Dactylis glomerata*	"	"
* Crested dogtail	*Cynosurus cristatus*	Jacques	(1940).
* Timothy	*Phleum pretense*	Doull	(1954).
* Sweet vernal	*Anthoxanthum odoratum*	Kelsey	(1958).
* Chewings fescue	*Festuca rubra* var. *fallax*	"	"
* Oats	*Avena sativa*	"	"
* Rape	*Brassica rapa*	"	"
* Browntop	*Agrostis tenuis*	Pottinger	(1961a).
* Maize	*Zea mays*	Kain & Barker	(1966).
* Tall fescue	*Festuca arundinacea*	Barker *et al.*	(1983).
* Paspalum	*Paspalum dilatatum*	Barker *et al.*	(1984a).
* Yorkshire fog	*Holcus lanatus*	Barker *et al.*	(1985).
* Phalaris	*Phalaris aquatica*	"	"
* Meadow fescue	*Festuca pratensis*	Pottinger	(1985).
* Poa annua	*Poa annua*	Prestidge *et al.*	(1986).
* Praire grass	*Bromus willdenowii*	"	"
*** Brassicas	*Crucifera* spp.	Lowe	(1956).
*** Clover	*Trifolium* spp.	Kelsey	(1958).
*** Lucerne	*Medicago sativa*	"	"

* Attacked by both larvae and adults

*** Attacked by adults only

Newly laid eggs are smooth-walled, shiny and elongate, rounded at the ends and oval in section. Their mean length is 0.95 mm and mean breadth 0.25 mm. They are a yellow-white colour but mature to olive-green (Morrison 1935; Blair and Morrison 1949; Kelsey 1958; Pottinger 1961a and b, 1977; and Power 1974). The first generation eggs appear at the end of August and peak in early October, while second generation eggs appear mid December, peaking in late January.

In the Auckland region May (1961) reported hatching of first instar larvae from eggs within 9 to 34 days, while Pottinger (1961a and b) and Buchanan (1966) working in Canterbury, reported 17 to 27 days and 17 to 21 days respectively. Upon hatching, the 1 mm long legless yellowish-white larvae bore through the upper epidermis of the leaf sheath and into the tiller. They then tunnel downwards and sever the innermost folded leaf and crown of the plant. Feeding tunnels 19 mm to 64 mm are common (Pottinger 1961a). Reputedly only one larva survives per tiller, even though empty egg shells suggest more larvae emerge (Pottinger 1961a and b). Upon exhaustion of the food supply, larvae can migrate in search of other suitable tillers by chewing exit holes approximately 1 mm in diameter and within 6 mm of the ground (Kelsey 1958; Pottinger 1961a and b). Duration of the larval stage has been estimated to be between 14 and 66 days (May 1961) and between 50 and 61 days (Pottinger 1961a). Power and Singh (1974), observing moults and counting exuviae, postulated four instars. This was confirmed by Goldson (1979a) using head capsule measurements and Dyar's Law.

Detailed taxonomic information on larval morphology is given by May (1966; 1977).

Mature larvae are 6mm long. They chew exit holes in the leaf and drop to the ground to prepare an earthen cell in which to pupate, usually within the top 13 mm of soil. They compact the soil around themselves into a smooth-walled cell 3 to 4 mm long and half as wide. Once the cell is completed, the larva becomes inactive and curls up into a crescent-shape, with the body shortening and becoming swollen in the thoracic region prior to pupation. Most larvae pupate within 6 days of dropping, but differences between 1 and 16 days were reported for this pre-pupal stage by Pottinger (1961a) and between 4 and 12 days by May (1961).

The pupae are exarate and less than 3mm long (Power and Singh 1974). At first they are a creamy-white colour, but darken to an ochreous red as development proceeds. Pupae are active until a few days before eclosion which occurs inside the cell. The teneral adults must then dig out to emerge (May 1961). Pottinger (1961a) observed that these weevils assume their normal dull grey colour after about 7 days, but this time varies extensively. The pupal stage is reported to be between 7 and 13 days duration by May (1961) and 8 and 24 days by Pottinger (1961a).

The emergent adult, due to its cryptic colour and small size (3mm long) is well camouflaged, remaining closely concealed during daylight hours in the crowns of plants and litter layer of dead leaves. It climbs to the leaf tips mainly at night to feed (Kelsey 1958; Pottinger 1961a and b; and Barker and Pottinger 1986).

The external morphology of adult ASW has been described by Hustache (1929), Blair and Morrison (1949) and Pottinger (1961a and b). Similarly, the reproductive morphology has been fully described by Goldson (1979a).

If unfavourable conditions are encountered the weevil is very capable of flying. Flight activity was reported by Kelsey (1958), Morrison (1959), and Pottinger (1961a and b; 1966) in the Canterbury region. Pottinger (1966) observed that the stem weevil flight period can extend from late August to late April. He also related flight activity to the meteorological pressure systems involved in changes from north-easterly through north-westerly to south-westerly air streams, when the air temperature was above 15°C, relative humidity below 64%, and wind speed less than 13 km per hour. His data also showed flight occurrence to be intermittent and of varying intensity, but often sufficient to reinfest insecticidally treated areas rapidly. Goldson (1981) added to this information and interpreted the flight as "migratory", and unrelated to sexual activity. Flight activity in the North Island, however, is very much reduced (Prestidge *et al.* 1983).

ASW completes two generations per year in most regions of New Zealand (Kelsey 1958; Pottinger 1961a), but three generations may occur in northern districts (May 1961). Egg laying commences in late August/early September by the adult over-wintering population. These develop into the "first summer generation" which begin emerging as adults in early December. These in turn produce eggs from mid December onwards, which give rise to the "second summer generation" from early March. This latter population of adults over-winter and produce eggs in the following spring (Kelsey 1958).

Pottinger (1961a and b) expanded Kelsey's 1958 work and provided detailed data on the bionomics of ASW. However, these studies allowed no conclusions to be drawn as to the mechanisms controlling the insects reproductive seasonality. Goldson (1979a and b) undertook a detailed three-year study to improve the understanding of ASW phenology, which involved regular dissection and examination of gonad condition. He demonstrated that adult weevils enter hibernatory diapause in early March and remain in this state until late July when reproductive activity resumes. Adults emerging in February may enter diapause immediately. The existence of reproductive diapause was further supported by an associated cessation of mating, increase in body fat and apparent drop in the haemolymph titre of juvenile hormone. Laboratory experiments indicated that a critical photoperiod induces diapause and an eventual reduction in sensitivity to photoperiod allows reproductive activity to resume (Goldson 1979 a and b, 1981, 1983; Goldson and Emberson 1980 and 1981; and Goldson, Emberson and Bickerstaffe 1981). Ahmad (1978) similarly noted that the weevil entered diapause from March to August in the Bariloche region of Argentina.

The rate of development of stem weevil populations is highly variable and dependent upon the time of year. In the coolest part of the reproductive season in Auckland, it can take the weevil up to 18 weeks for development from egg to adult, compared to 5 weeks under optimal field conditions (May 1961). This latter figure conforms to the findings of Power and Singh (1974) who reared the pest in artificial media at 26°C. Pottinger (1961a) found the average time to be 15 weeks.

Damage.

Damage is caused by feeding activities of both adults and larvae. Ryegrasses are the preferred host plants. Adults feed externally on foliage whilst larvae tunnel within vegetative and flowering tillers. The larval stage is considered the most destructive. In the process of feeding within vegetative tillers, larvae cause the tillers to die slowly outward from the innermost rolled leaf and growing point. These leaves turn yellow, wither and finally die. Feeding damage can be recognised by the brownish colour surrounding the tunnels and the general bruised appearance of a tiller. Tiller death usually results from either severing the plant's vascular tissue, thus stopping the water and nutrient flows between roots and leaves, or through the destruction of the apical meristem. Similarly, in flowering tillers, death results from severing vascular tissue to the inflorescences or from mechanical breaking of the culms. The latter follows from larval attack weakening the stems at nodes or internodes.

Collapse of nodal tissue due to larvae "girdling" a node is often followed by a "stem-break", where the tiller snaps off because of wind or seedhead weight. Flowering tillers that die from weevil damage before seed is set result in the production of "whiteheads." This is again due to severing the vascular supply (Morrison 1935, 1938; Blair and Morrison 1949; Kelsey 1958; Pottinger 1961 a and b, and Timlin 1964). The effect of larval ASW on maize has been summarised by Kain and Barker (1966).

In established pastures adult ASW feed nocturnally all year round. For most months this feeding is inconspicuous and insignificant for plant survival unless complicated by stressful conditions of drought and over-grazing when tiller replacement is checked (Pottinger 1961a and 1977). However, quite the contrary occurs in immature crops. Lowe (1956 and 1958) and Whatman (1959) noted that the weevils attack seedling brassica leaves by chewing through their stems. Similarly, seedling grasses can be totally destroyed (Pottinger 1961a and b, and 1977; Trought 1976), especially when weevil populations exceed 20,000,000 per hectare (Pottinger 1977). In Canterbury, where regular flights occur from spring to late autumn, newly sown pasture can be quickly infested and population increases of up to 100 weevils / m^2 have been recorded after a single dispersive flight (Pottinger 1977).

When adults feed, leaf tissues are scraped off with the deeply toothed mandibles leaving only the cuticle and sometimes the broken veins. This shows up characteristically as whitish windows and curled thread-like veins. Feeding is generally initiated at the leaf tip and progresses downward (Blair and Morrison 1949; Kelsey 1958; May 1961; and Pottinger 1961a).

Pest Status.

As mentioned above, usually only one larva occurs in each tiller, yet each larva during its development may destroy three to five tillers plus a number of replacement buds at the base of tillers. This severely reduces production, but because it is insiduous, it can easily be concealed by replacement growth and can go un-noticed. For example, tiller mortality of up to 50% may not be readily apparent due to dilution by new growth (Pottinger 1977). However, damage becomes very noticeable in dry summers when tiller replacement is checked by lack of water and whole plants may die. Any condition which places the plants under stress, such as drought, over-grazing and general pasture mis-management will accentuate ASW damage.

Kelsey (1958) was first to publish results implicating the importance of ASW as a pest. In the area studied, 98% of vegetative tillers and 6% of flowering seed-heads were damaged. Pottinger (1961a) found up to 64% of the inflorescences in "Grasslands Manawa" ryegrass seed crops had been damaged. Timlin (1964) reports tiller damage in short-rotation ryegrasses as high as 84%, while Prestidge *et al.* (1982) reported 85% tiller mortality in grazed Manawa/white clover pasture in the Waikato. Similarly, a survey of 28 established pastures in the Waikato/Bay of Plenty in 1980 showed that ASW larvae commonly infest up to 25% of grass tillers and sometimes up to 90% over the spring/summer period (Barker *et al.* 1981). All these authors noted that short rotation ryegrasses were more susceptible than perennial ryegrasses.

Measurement of tiller mortality is a good index of ASW damage, but offers no measure of actual production losses. Much depends on the recovery of pasture after attack and this is very dependent on the season and management of the pastures concerned. Kain *et al.* (1977) estimated total herbage production losses from three ryegrass cultivars ("Grasslands Ruanui", "Ariki" and "Nui") to be between 11% and 13%. This peaked at 29% in summer, when the grass was most for stock. Similarly Goldson and Trought (1980) demonstrated the elimination of grasses by ASW in an untreated plot compared to an insecticidally treated counterpart. In terms of dry matter of grass the insecticide treated plots yielded a mean at 87.5 g/m^2, compared to 26.6 g/m^2 in untreated plots. This corresponds to a 230% increase in ryegrass production over the 12 week period. Untreated plots reverted to clover domination. Likewise Barker *et al.* (1983 and 1984) related the effect of ASW on productivity of grasses in the Waikato. Application of systemic insecticides increased the yield of grazed "Nui" ryegrass pasture by 17% over the spring and summer period. Similarly in autumn-sown plots of "Manawa", "Ruanui", "Nui", and Yates "Ellett" ryegrasses, sown with other grass species such as browntop, cocksfoot, paspalum, phalaris, and tall fescue, between 15% and 91% higher yields were obtained under insecticide protection during the first spring-autumn period.

In monetary terms, the pasture losses due to ASW have been estimated to be in excess of $150,000,000 per annum (Pottinger pers. comm. 1985).

Control

Insecticidal

Because ASW is recognised as a serious pest of pasture grasses throughout New Zealand much research has been conducted upon chemical control. Early researchers include Lowe (1956, 1958), Kelsey (1958, 1959), Whatman (1959), Pottinger (1961a), Nielson (1964, 1966), Buchanan (1966), Bahadur (1970), Forgie (1974), Welsh *et al.* (1974), Mackay and Rowe (1974), Trought (1976), Watson and Wrenn (1978), and Prestidge *et al.* (1983).

In established pastures only oxamyl and chlorpyrifos are registered for control of ASW larvae and adults respectively. Carbofuran can be drilled with seed to provide seedling protection. Oxamyl is recommended for application at 0.48 kg a.i./ha, and chlorpyrifos at a rate of 0.6 kg a.i./ha. (Pottinger *et al.* 1984). Other chemicals (granules of PP 993 and terbufos) are effective in reducing ASW populations but do not increase pasture production (Prestidge *et al.* 1984b). In comparison, a dual summer treatment with applications three weeks apart of oxamyl at 0.48 kg a.i./ha gave 78% control of ASW larvae (Prestidge *et al.* 1984b), and increased tiller survival by 149% and ryegrass herbage production by 51% (Prestidge *et al.* 1983).

To find chemicals with activity and persistence equal to or better than oxamyl or chlorpyrifos, but at reduced costs, Pottinger *et al.* (1984) screened the activity of 24 chemicals against the adult weevils in bioassays. All three chemicals, oxamyl, carbofuran and chlorpyrifos, registered for control of ASW were effective at 1 kg a.i./ha. against adults. Oxamyl and chlorpyrifos were not as effective as carbofuran when applied at 0.5 kg a.i./ha., and all were ineffective when applied at 0.25 kg a.i./ha. Carbofuran, isazophos, oxamyl, and phosmet, when applied at lower rates of 0.5 kg a.i./ha. and deltamethrin (0.025 kg a.i./ha.) all gave equivalent or better control for longer periods compared to chlorpyrifos. At current prices, phosmet and isazophos are cost competitive with chlorpyrifos. Carbofuran is effective when applied at 0.5 kg a.i./ha. but unfortunately, surface applications are not permitted on pasture. Azinphos methyl is effective at 1 kg a.i./ha. Both oxamyl and methiocarb were not cost competitive on the basis of the preliminary trial.

The results clearly showed that the level of mortality and persistence was related to the rate of each of the chemicals applied. However, in view of their toxicity to weevils, persistence, use against other pasture pests, and cost, isazophos, phosmet, and deltamethrin show particular promise as alternatives to chlorpyrifos, the current recommended control for adult ASW (Pottinger *et al.* 1984).

While there are chemicals registered for effective control of ASW, excessive costs prohibit their widespread usage ($70 - $120 / ha. (East and Pottinger 1984)). The latest references point to a current line of research where cheaper alternatives are being considered, and this is continuing, as the utilisation of cheap and efficient insecticides is essential to protect graminaceous crops in New Zealand,

Cultural

Cultural control methods have also been developed, but mainly before the full impact of endophyte was realised. Many are still applicable, especially those relating to the time of sowing the susceptible annual ryegrass cultivars. Morrison (1935) was first to recognise the potential for the avoidance of ASW by co-ordinating sowing times with the various phases of the pest's life cycle. Similarly Pottinger (1961a) and Batten (1964) suggested sowing pastures in autumn to allow sufficient time for their establishment before ASW attack, as the mature plants may "tolerate" ASW. Goldson and Penman (1979) predicted sowing times to be the end of the first week of March in Canterbury for minimal weevil damage. However, the concomitant predictability of the onset of ASW reproductive activity and the weevil's diapause seasonality, as proposed by Goldson (1979a), enable the selection and timing of insecticidal applications to be more precisely determined. Because of this predictability ASW has been considered suitable for computer modelling (Goldson *et al.* 1982). This is a logical extension of increased phenological understanding leading to more effective use of management techniques (Tauber and Tauber 1976).

Gaynor and Hunt (1982 and 1983) and Hunt and Gaynor (1982) examined environmental factors such as moisture stress and soil fertility in relation to ASW control. In field experiments they showed adult ASW preferred plants grown under high nitrogen and water levels, which was reflected in greater oviposition, larval numbers, and damaged tillers. This indicates that managerial practices can markedly affect the level of ASW damage. In fact, any factor that limits the growth of susceptible grasses in summer increases the level of

ASW damage substantially. Such factors as undergrazing, overgrazing, and hay cropping, all reduce tillering and as a consequence, increase the level of ASW damage (Barker *et al.* 1985).

The cultural practice of introducing or encouraging pest resistant pasture plants is discussed later.

Biological

Few pathogens of ASW have been described. In Argentina, a nematode, probably in the family Allantonematidae was reported from the haemocoeles of adult weevils, and some "sporozoans" (Protozoa) have been noted in the gut tissue of some adults (Lloyd and Ahmad 1972). Malone *et al.* (1984) investigated some protozoan pathogens of ASW in New Zealand. They found two microsporidian pathogens (phylum Microspora) and one neogregarine (phylum Apicomplexa) infecting ASW adults and larvae. The first microsporidian was tentatively identified as an *Orthosoma* spp., but more recently was reclassified as a completely new pathogen, and named *Microsporidium itiiti* (Malone 1985a). It does not appear to be particularly virulent since heavily infected weevils display no external symptoms and remain quite active. Some weevils, however, had deformed ovaries full of *M. itiiti* spores. This reduces fecundity as it shortens the period of egg-laying (Malone 1987). Transmission occurs mostly by contamination of food with spores from frass or cadavers (Malone 1985b). *M. itiiti* is widespread, occurring in every area sampled, with incidence of infection as high as 67%. The second microsporidian was tentatively identified as a *Nosema* spp., and is not as widespread, being found so far in only 5 specimens. The neogregarine protozoan remains unidentified, and is also uncommon, being found in only two specimens.

A further microsporidian which is commonly found infecting pasture pests, *Vavraia oncoperae*, is also occasionally found in ASW (Malone *et al.* 1987). Its effects on ASW in the field are unknown.

A fungal pathogen, *Beauvaria bassiana*, has been noted in the Waikato (Barker pers. comm. 1985), at Palmerston North (pers. obs. and Gaynor pers. comm. 1984) and at Canterbury (Goldson pers. comm. 1984). It has a very broad host range covering most insect groups, including Curculionids, and has a world-wide distribution (Deacon 1983). Research is continuing into this pathogen in the Manawatu (Hunt pers. comm. 1986).

No bacterial or viral pathogens have been recorded for ASW (Malone pers. comm. 1987).

Loan and Lloyd (1974) published a full description and biological account of a new species of Braconid wasp which is found in South America, and named it *Microctonus hyperodae* (Loan), [Hymenoptera: Braconidae, Euphorinae]. This wasp specifically parasitises ASW. The D.S.I.R. is currently investigating importation of this species, (D. Gaynor pers. comm. 1985).

A further parasitic wasp showing potential as a parasite is *Potasson atomarius* (Brethes), [Hymenoptera: Mymaridae] a parasite of ASW eggs in South America (Ahmad 1977 and 1978). Ahmad (1977) mentions other antagonists which he considers unimportant in regulating ASW populations. These are *Sericophanes obscuricornis* Popp. (Miridae); *Nabis punctipennis* Blanch. (Nabidae); and *Philonthus* sp. (Staphylinidae), which are predaceous on stem weevil eggs, plus *Pterostichus aereus* (Dej.); *Pt. unistriatus* (Dej.); *Barypus clivinoides* Curt.; *Cnemalobus gavi* Putz.; *Metius blandus* (Dej.) and *M. malachiticus* (Dej.) (Carabidae), which are predaceous on adult ASW.

It has been noted that ASW adults make a major contribution to the food supply of starlings (*Sturnus vulgaris*) throughout the year in mid - Canterbury (Lobb and Wood 1971). No doubt other bird species, while not controlling the insect, contribute to decreasing the numbers of ASW.

Plant Resistance

The use of cultivars resistant to insect pests is becoming more common in plant breeding programmes as its importance and potential are realised. Plant resistance is a very cheap form of pest control. Resistant plants do not pollute, affect beneficial organisms, or require periodic applications. It is therefore a particularly attractive approach to pest problems in low value crops which may not generate sufficient profit to justify more costly measures e.g. most pastoral farming. The main objective of the present research was to examine the interrelationship between ASW and a number of ryegrass host-plants in an attempt to elucidate some of the mechanisms of plant resistance. Integration of resistant plants with other control methods would hopefully reduce the impact of ASW as a pest in New Zealand.

Beck (1965) defines plant resistance as the "collective heritable characteristics by which a plant species, race, clone, or individual, may reduce the probability of successful utilisation of that plant as a host by an insect species, race, biotype, or individual". To understand the mechanisms of plant resistance to insects, it is important to consider their different categories and modes of operation through component responses of insects and plants. The mechanisms currently widely recognised were originally proposed by Painter (1951). These are (1) preference/non-preference for different plants for oviposition, food, and shelter; (2) antibiosis, affecting insects' survival, development and egg production on plants; and (3) tolerance in plants involving repair and regeneration of their damaged tissues.

In order to identify the plant characters which would actually impart resistance or susceptibility to plants against insect species, it is necessary to consider the modes of operation of the above-mentioned mechanisms. The first two categories involve certain responses of the insect determining their establishment on plants, whereas the third category involves the responses of plants to insect attack.

On the basis of the reviews by Painter (1951), Thorsteinson (1960) and Beck (1965), Saxena (1969; 1974a and b) recognised six responses of an insect species which determine the establishment of its populations on plants. These are 1) orientation,, determining the insect's arrival on or avoidance of a plant in response to its attractant or repellent or neutral stimuli; 2) feeding responses, determining the quantity of food ingested from the plant; 3) metabolic responses involving the utilisation of the ingested food and determining the insect's nutrition; 4) development of the insect, if in the larval stage, determined by the quantitative food-intake and nutrition; 5) egg-production in the adult stage, determined by the quantitative food-intake and nutrition; and 6) oviposition.

Orientation, feeding and oviposition responses by the insect are behavioural parameters which are involved in the "non-preference" type of resistant mechanism, in which a plant has characters that fail to elicit these responses or inhibit them. The metabolic responses of insects are involved in the "antibiosis" type of mechanism of resistance in a plant, which occur if the latter provides inadequate nutrients or metabolic inhibitors to cause failure or limitation of larval development, survival and egg production in the adult stage. Differentiation of insect behaviour into one or other of these resistant mechanisms is not always precise and clear cut as they may operate simultaneously. However, it is behavioural responses of ASW and plant characters which determine the susceptibility or resistance of different ryegrass cultivars which are primarily considered in this thesis.

Orientation

In order to understand the mechanisms of insect resistance in plants and the characters involved, the first step is to compare the behavioural responses of an insect to a given set of resistant and susceptible plant species or varieties. This would show the relative importance of different responses of the insect to the plants in relation to their resistance or susceptibility and provide the basis for examining the plant characteristics involved (Saxena 1985).

The initial step in the host-plant selection process is the orientation of an insect toward a potential host plant. There are two main possibilities (Kennedy 1965; Saxena 1969). Either the insect makes oriented movements towards suitable plants (by visual and/or chemical stimuli, i.e. attractants), or its movement is random and host-plant location is by chance. The latter must involve some arrestant so that the insect stays on a suitable plant, but takes off again from one that is unsuitable. Plant recognition after contact must therefore take place. There is also the possibility that some non-host plants may be repellent so that the insect never makes contact with them.

One of the best examples of chance location is that of some aphids which seem to land on plants at random. This is followed by either acceptance and initiation of feeding, or take off and a repeat of the process (Kennedy 1976). Also, many small insects and weak fliers are dispersed passively by the wind, controlling only the initiation and to some extent the duration of their flight (Hawkes 1973). Many insects, however, simply land on plants because the plant is abundant or large or conspicuous in some way. Feeny (1976) refers to the species which are highly susceptible to discovery as "apparent" plants.

Visual orientation to host-plants from a distance is exemplified by many aphids and whitefly. Light reflected from green leaves contains a distinct peak in the yellow portion of the spectrum, and these insects are attracted to such yellow-reflecting surfaces (Kring 1972). Colour or intensity of light reflected from the surface of cabbage leaves affects host selection by *Brevicoryne brassicae* (Radcliffe and Chapman 1965 and 1966). Another example is the boll weevil (*Anthonomus grandis*) which much prefers green cotton when red and green plants are grown together (Stephens 1957; Reddy and Weaver 1975).

Form perception probably elicits certain generalised behaviour patterns such as landing. For instance, locusts and grasshoppers were shown to be attracted to vertical striped patterns, but not to horizontal contrasts (Mulkern 1969). In the genus *Rhagoletis* (Diptera: Tephritidae), foliage colour plus tree shape and size play roles in the discrimination between hosts and non-hosts (Boller and Prokopy 1976). Of the visual properties of plants (colour, pattern, and dimensions), it seems that their spectral differences elicit more behavioural responses than the patterns or dimensions (Prokopy and Owens 1983).

Probably the best examples of orientation come from reactions to phytochemicals. Plants are very rich in chemicals which are apparently not directly connected with the normal metabolic processes of photosynthesis, respiration and growth, but are attributed to have mostly defensive functions. These are called "secondary" plant chemicals (Fraenkel 1959, 1969) or allelochemics (Whittaker 1970). They include alkaloids, steroids, phenolics, saponins, tannins, resins, essential oils, various organic acids and other compounds. Harborne (1977) lists over 30,000 known chemical structures for such secondary compounds. The phytochemicals primarily involved in orientation behaviour are volatile and provide olfactory stimuli perceivable at a distance from a plant. For example, the important phytochemical attractants for ovipositing cabbage root flies (*Delia brassicae*) are the "mustard oils", particularly allylisothiocyanate (Wallbank and Wheatley 1979). Attraction may occur over distances of up to 24 metres (Hawkes 1974, 1975).

Orientation behaviour itself begins after randomly moving insects enter an odour plume. They then may turn upwind (sometimes at increased speed) using optomotor stimulation and thus show positive anemotaxis (Kennedy 1977). As long as the insect remains in the odour stream, direct or weakly zigzag flight paths are maintained until the insect overshoots the odour source. At this point the insect begins a series of randomly directed turning movements which usually brings it back into the odour stream. When the insect comes into the proximity of an odour source, the concentration gradient becomes much steeper, and in principle becomes measurable, thus allowing chemotactic orientation (Kennedy 1977). High concentrations of an attractive odour may also inhibit locomotion (arrestant effect), and consequently induce the flying insect to land (Douwes 1968). At close range, the insect may obtain additional olfactory information, especially with respect to differences between individual plants. For example, some plants in a cabbage field are more attractive for oviposition by *Pieris brassicae* because they contain higher than average amounts of volatile allyl nitriles (Mitchell 1977). It must be emphasised, however, that the physiological state of the insect is important. When male and barren female cabbage root flies are released near a brassica crop they disperse randomly and do not appear to be influenced by crop odour (Finch 1978). Similarly, only ovipositing fruit flies will respond to the shape or contour of potential oviposition sites (Boller and Prokopy 1976). Similar responses involving a wide range of chemicals, both separately and in combination, have been recorded for a number of different insects (Dethier 1976).

Orientation to potential host-plants and discrimination between host and non-host requires a highly developed sensory system. Usually the insect must respond to the odour of a plant located in a stand of mixed vegetation. Specific plant "odours" are seldom single compounds, but are usually complexes of several volatile substances. In some cases, however, one component of the plant volatile complex is predominant with respect to insect orientation. This is called a "token stimulus" as it signals the presence of the host plant. Such substances as terpenes, flavonoids, alkaloids, and nitriles act as token stimuli.

Orientation to the host plant may be still more complex by involving a number of complementary stimuli. For example, attraction of the leafhopper *Empoasca devestans* to its host-plant cotton, involves light intensity, colour, humidity, and volatile chemicals. The non-specific stimuli such as background light intensity and colour act over a longer distance than the more host-specific chemical stimuli (Saxena and Saxena 1975).

Oviposition and Feeding.

After arriving on a potential host-plant, acceptability to the insect for oviposition and/or feeding is largely determined through chemical cues, but physical factors are also of paramount importance in the recognition phases that immediately precede oviposition and/or feeding. These characters are the morphological and anatomical features of plants which may provide contact stimuli or mechanically facilitate or prevent feeding or oviposition by insects. For example, many plant surfaces are not smooth, but clothed in minute epidermal hairs or trichomes which occur in a multitude of different forms. Such pubescence interferes with insect attachment to the plant, and therefore to oviposition, feeding and/or ingestion (Levin 1973). Pubescence may also provide a mechanical barrier to insect mouthparts and/or ovipositor from reaching the plant tissue underneath the trichomes. For example, the cotton boll weevil (*Anthonomus grandis*) lays far fewer eggs on pubescent (resistant) varieties than on glabrous (susceptible) varieties of cotton (Stephens and Lee 1961).

Similarly, leaf pubescence in wheat greatly reduces oviposition by the cereal leaf beetle (*Oulema melanopus*) on the resistant varieties as compared to that on susceptible varieties (Gallun *et al.* 1973; Webster *et al.* 1973).

Some trichomes can actually trap or impale insects. The trapped insects stop feeding and struggle, frequently dying from dehydration and starvation before they can escape (Pillemer and Tingey 1976). In some cases trichomes possess associated glands that exude secondary plant metabolites. The effect of glandular trichomes may depend on the nature of the exudate. It may be composed of allelochemics such as alkaloids or terpenes (Johnson 1975). Such toxic substances may kill insects on contact or act as repellents (Thurston 1970). In some plants sticky exudates glue the insects' legs and impede locomotion (Gibson 1971), or dislodge the insect from the plant (Gibson 1976).

In many instances, however, trichomes are beneficial. The insect ovipositor generally bears mechanoreceptors, and in most cases tactile stimuli seem to be the only sensory information relayed by the ovipositor (Beck and Schoonhoven 1980). However, some species require relatively glabrous surfaces, whereas others prefer heavily pubescent oviposition sites. Other surface characteristics are also frequently important. The diamond back moth, *Plutella maculipennis*, prefers an oviposition substrate with small crevices and cavities (Gupta and Thorsteinson 1960). With the cotton bollworm, *Heliothis zea*, glabrous cotton strains of *Gossypium* (cotton) species are less favourable substrates than pubescent strains for oviposition (Lukefahr *et al.* 1971, 1975). This could be due to the pubescent leaf surface providing a better foothold which facilitates oviposition (Callahan 1957), or the stimulation of mechanoreceptors on the ovipositor. In some cases the surface shape may have tactile significance. For example, the weevil *Ceutorrhynchus maculaalba* oviposits in young seed capsules of poppies; the convexity of the seed capsule was shown to be of decisive importance in host selection. Presumably the proprioceptors in the weevil's legs perceive the degree of convexity (Saringer 1976). In contrast to oviposition, food selection is usually less affected by tactile factors. This does not mean, however, that the physical characteristics of plant tissue do not play a role in host selection and in the successful utilisation of a host plant. In numerous cases utilisation is prevented by physical barriers such as hard spines, dense pubescence, and sclerenchymised leaf edges. However, the numerous mechanoreceptors located on insect mouthparts are more functional in the mechanics of biting, chewing, and swallowing (Beck and Schoonhoven 1980).

In direct opposition to the effects of pubescence, a very smooth surface of a leaf may present problems of obtaining secure anchorage, especially if compounded by the effects of wind and rain. It has been noted, for example, how large numbers of aphids are dislodged from herbaceous plants and trees during heavy rain or wind (Strong *et al.* 1984).

The cuticles of most vascular plants are covered with a thin layer of largely hydrophobic constituents, collectively called waxes. A detailed analysis of the surface waxes of some of the common plants is given by Martin and Juniper (1970). These waxes function primarily in the mechanisms of water balance of the plant, but also contain substances that inhibit pathogens and interfere with insect attacks. Surface waxes of certain plants seem to be inhibitory to some herbivores (Anstey and Moore 1954), but excitatory to others (Thompson 1963).

Any modification in the morphological structure of plants may result in altered fitness to herbivores. Such variations include thickening, toughening, or hardening of plant tissues caused by lignification, silification, or calcification, the rapid proliferation of cells triggered by insect injury in the wounded tissues, plus the anatomical adaptations of non-specialised organs and protective structures. The relationship between these morphological variations and plant resistance to insects has been considered by Norris and Kogan (1980).

The examples above emphasise physical defences of plants against insect depredation. Physical factors, however, are rarely unique to a single plant species. It is chemical characteristics which show a higher degree of specificity. It is not surprising, therefore, that chemical stimuli play a major role in host plant selection for oviposition and feeding. The volatile chemicals frequently involved in orientation to plants from a distance (olfactory stimuli) are also known to stimulate biting, probing, and oviposition after the insect is in physical contact with the plant. However, the final recognition process leading to acceptance or rejection is usually mediated by non-volatile chemicals acting on contact chemoreceptors. There is wide variation in the way insects perceive and react to these chemicals, and it is difficult to generalise. Some insects are "arrested" by chemical stimuli received through contact chemoreceptors situated on the fore-tarsi, mouthparts or ovipositor, others immediately take a test bite. Biting brings the chemoreceptors on the mouthparts into direct contact with the plant sap. Special movements by the insect may intensify the chemical stimulation; examples include the tapping or scraping motions of the forelegs of many butterflies as they prepare for oviposition (Fox 1966; Ma and Schoonhoven 1973), and the drumming of maxillary and labial palps by locusts (Edwards and Wratten 1980).

Surface testing by touching or piercing with the ovipositor, or by biting and probing with the mouthparts, is in response to chemical factors that act as "incitants" which are occasionally the same as the odour factors that attracted the insect to the plant. If the stimuli received upon initial testing identifies the plant as an acceptable host, feeding or oviposition proceeds. These chemical factors are "stimulants." If stimuli received on initial testing indicate an unacceptable plant, the behaviour pattern is interrupted, and the insect abandons the plant. Such stimuli are "deterrents." Whereas attractants, repellents, and many incitants are olfactory substances, stimulants and deterrents are usually gustatory, representing the non-volatile chemicals (Beck 1965). With respect to feeding stimulants, they have been reported to be present in cotton plants to stimulate feeding in the boll weevil, *Anthonomus grandis* (Maxwell *et al.* 1963, 1969). One of the chemicals involved in eliciting this response is gossypol (Maxwell *et al.* 1967). For chemicals inhibiting feeding, certain unidentified constituents of cotton plants have been reported to act as feeding deterrents for the boll weevil (Maxwell *et al.* 1969). For further references on feeding see Chapman (1974).

With respect to ovipositional responses to non-volatile chemicals, cotton plants have again been reported to posses chemicals which elicit oviposition in the boll weevil (Maxwell *et al.* 1969). While all the chemicals responsible for this are not known, one of them appears to be gossypol. Incorporation of this chemical in an artificial medium stimulates more egg laying by the weevil than in its absence (Maxwell *et al.* 1967). With reference to ovipositional inhibitors or deterrents, these have also been reported in cotton plants (Maxwell *et al.* 1969). For more specific information on non-volatile chemicals and the host-plant selection process see the reviews by Beck (1965), Chapman (1974), Hedin *et al.* (1974), Kogan (1977), Harborne (1977), Jermy and Szentesi (1978), and Norris and Kogan (1980).

Many important feeding stimulants are general nutrient substances such as sugars and amino acids, rather than host plant specific compounds, and there has been a tendency to ignore the role of these substances in the behavioural processes of host selection. Several carbohydrates, most notably sucrose, glucose, and fructose, stimulate feeding in many phytophagous insects. Sucrose is one of the most potent and universal feeding stimulants known. In addition, several amino acids, sterols, phospholipids, and a few other biochemicals of general occurrence are known to influence feeding behaviour. Many of these substances have great nutritional importance. Through the insect's reaction to such key nutrient compounds, there is a connection between the insect's nutritional requirements and its host selection behaviour. It seems likely that token stimuli act as feeding incitants, whereas generally occurring compounds, such as sucrose, act as feeding stimulants. For example, sinigrin (the principal crucifer token stimulus) may incite biting by cabbage worm larvae, but continued feeding is determined by the presence of feeding stimulants (sucrose and others) in the plant tissues being eaten (Beck 1965). Synergistic interactions have also been demonstrated in which a mixture of token stimuli and feeding stimulants has a much greater than additive effect on feeding (Gothilf and Beck 1967).

In nature, an insect is never exposed exclusively to either token stimuli or to general compounds, but always to different mixtures of these factors. Host-plant selection is therefore not based on simple sign stimuli, but rather on a complex chemical pattern, a "Gestalt", which must fit into a species- specific "innate releasing mechanism" to evoke feeding or ovipositional behaviour.

This complexity can be further amplified when one considers the mutualistic relationship between endophytic fungi (Ascomycetes: Clavicipitaceae: tribe Balansiae) which infect many wild and domesticated grasses (Diehl 1950). Alkaloid chemicals produced through the plant/fungus interaction are postulated to be responsible for toxicity problems that arise when livestock graze on infected pastures (Bacon *et al.* 1975, 1977; Porter *et al.* 1979a, b, 1981; Hoveland *et al.* 1980; Fletcher and Harvey 1981; Mortimer 1983; Jackson *et al.* 1984). In addition, infected plants have also been shown to deter or negatively affect a variety of insect pests. Of relevance to this thesis is the association between the endophytic fungus *Acremonium lolii* Latch, Christensen and Samuels (Latch *et al.* 1984) and its host-plant *Lolium perenne*, in relation to the resistance of the study insect and the animal disease of ryegrass staggers. Further reference to this association appears in the relevant sections of each chapter.

CHAPTER 3

ORIENTATION

INTRODUCTION.

Orientation to potential host-plants is the first phase in the establishment of an insect upon a host plant. Orientation may be a result of the insect being attracted to the host plant or repelled by a non host. Host-plant location may also be due to chance, in which case some form of arrestant is necessary once the host is reached. The finding of a host-plant by chance may simply be due to its abundance i.e. its "apparency" (Feeny 1976). Associated with this are arrestants which may be tactile, olfactory, gustatory, or any combination of these. Attractants and repellents may be either visual, olfactory, or combinations of both. Whatever the modality, meaningful orientation experiments are difficult to perform and interpret in the laboratory, and even more difficult to relate back to the natural environment. The literature on host-plant selection reveals that the matter of locating host plants in the environment was not so much neglected, but subordinated, in terms of research effort expended, to other aspects of the host-plant selection process. That this relative neglect occurred is hardly surprising considering the enormous difficulties attendant upon observing the behaviour of individual flying insects in their natural environments. Extrapolations from laboratory conditions to the natural environment must therefore be regarded as highly speculative.

As far as the orientational responses of ASW are concerned, visual discrimination between susceptible and resistant host-plants may be of limited importance because the weevil is primarily nocturnal (Kelsey 1958; Pottinger 1961a and b). However, as ASW infests virtually every improved pasture in New Zealand (Pottinger 1985), and daylight dispersal flights have been recorded in the South Island (Kelsey 1958; Morrison 1959; Pottinger 1961a, 1966 and Goldson 1981a), the initial attraction to potential host-plants may involve visual cues, and so these must not be overlooked. An experiment was therefore set up to investigate the orientational responses of adult ASW's to distinctly coloured nutrient agar plugs. The amount of feeding upon each plug was used as an indication of preference.

The olfactory responses of the weevils were investigated using an olfactometer similar to that of Vet *et al.* (1983). This design has four distinct odour fields allowing freedom of movement for the insect to explore them without being restricted to an initial and only choice, as is often the case with Y or T - tube olfactometers. The experiments involved testing the olfactory responses of adult ASW to various combinations of a susceptible host plant (Grasslands Nui perennial ryegrass **without** endophyte), a resistant host plant (Grasslands Nui perennial ryegrass **with** endophyte), and blank tubes without plant material (see Chapters 4 and 5 for details of resistant and susceptible host-plants with respect to endophyte). The naturally emitted volatiles from the ryegrass tissues (both endophyte-positive and endophyte-free plants) were collected and identified by gas chromatography and mass spectroscopy.

Dethier (1963) and Wigglesworth (1950) report that in many insects the antennae are the principle site of the olfactory receptors. Also, it has been pointed out that the distal segments of the antennae often contain the greatest concentration of these receptors (Dethier 1947, Anderson and Ball 1959). If olfaction is the weevil's primary sensory modality for orientation to host plants, then extirpation of their olfactory sensilla should abolish any such response. Four experiments in which the whole antennae or antennal segments were removed were conducted to test the weevils' orientation between susceptible and resistant host plants in both olfactometer and petri dish tests.

MATERIALS and METHODS.

Collection of Adult ASW.

Collection of adequate numbers of ASW adults was achieved at night using either a muslin net to sweep feeding weevils off grass or an open-ended bin dragged through grass. The pasture sampled was a weevil-infested 8 to 12 year old "Nui" ryegrass/clover paddock at D.S.I.R. Palmerston North.

Visual Orientation.

Two discs of one centimetre diameter which contained two drops of commercial food colouring, 4% Davis agar, 4% cellulose powder, 5% sucrose mixed in 90 ml of distilled water were presented to an adult weevil placed in a 15 cm diameter petri dish. Ten replicates were used, with each test running for 72 hours at 16°C with a LD 16: 8 hour photoperiod. Weevils were left for 24 hours without food or water prior to the experiment. The amount of feeding on each coloured disc was scored visually on a 0 - 3 scale (0 - no feeding, to 3 - large amounts of the disc shredded). The feeding scores were analysed using the Wilcoxon signed-rank test (Siegal 1956), to determine preferences. Five distinct colours were used: red, green, blue and yellow, with the blank white. A Pye automatic SP8-400 uv/vis spectrophotometer was used to determine the spectra of the colours. The experiment was arranged so each colour was compared with every other colour an equal number of times. According to the manufacturer, the food colourings are tasteless (to humans), so hopefully gustatory discrimination by the insects should be minimal.

To see if any flight behaviour was initiated (such as the lifting of the elytra and expansion of the wings), randomly selected insects within the petri dishes were observed during the first 16 hour light period.

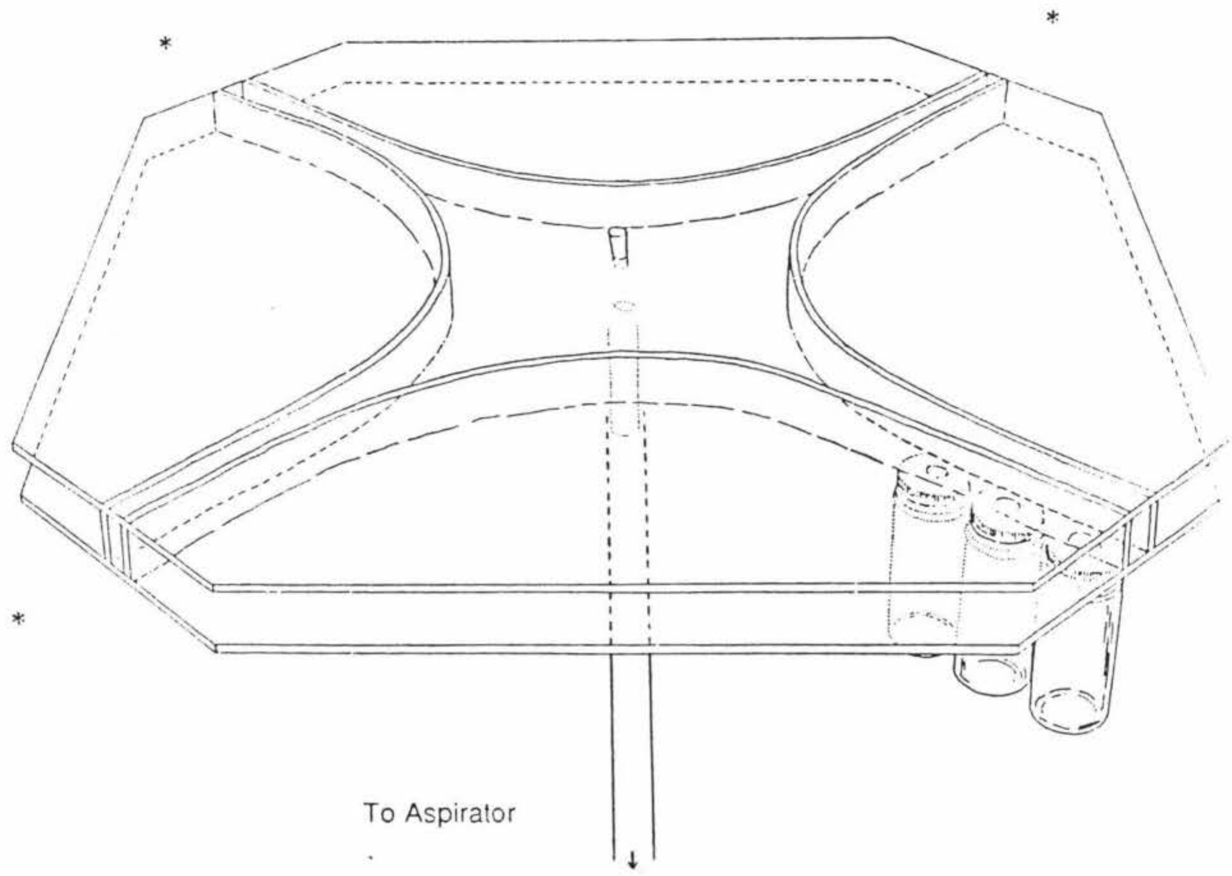
Olfactory Orientation.

The olfactometer is shown in Figure 1. The exposure chamber roof and floor was made from two parts of transparent 3 mm diameter perspex. The four pointed star-shape of the exposure chamber sides was constructed of four perspex crescents (90° arc, radius 40 cm) glued to the base with a silicon rubber sealant glue (Ados RTV Silicone Sealant). Each point of the star runs into a 5 mm (inside diameter) arm, covered with insect-proof netting. The perspex roof is held in place with four clamps and sealed with a teflon tape gasket.

Four odour fields were created in the chamber by sucking air out through a hole in the centre of the floor which is covered by insect-proof mesh. Insects were introduced through a corresponding centrally placed hole in the roof, which was later plugged with a small cork.

Air movement through the apparatus was provided by means of a 20 litre aspirator. Flow rate was regulated with a screw clamp to give an overall air speed of approximately 4 cm per second through the entire apparatus, calculated by a 10 ml bubble gauge flowmeter.

Figure 1



Perspective View of the Olfactometer.

Air is drawn through the exposure chamber equally over the four odour sources (only one is shown, the rest are represented by *) and flows towards the centre of the chamber and out through the central lower hole by aspiration.

A set of three 50 ml glass vials were connected to each arm of the chamber. The vial nearest to the chamber served as a pitfall trap and the second vial contained the odour source. The outer vial contained distilled water over which the incoming air was passed to create a high, uniform humidity.

Air is drawn through the exposure chamber equally over the four odour sources (only one is shown, the rest represented by *), and flows towards the centre of the chamber and out through the central lower hole by aspiration.

The experiments involved testing the olfactory responses of adult ASW to odour from a known host-plant: the susceptible "Nui" perennial ryegrass without an endophytic fungus and a resistant variety of "Nui" perennial ryegrass which contains the endophyte, plus blank tubes in various combinations. Weevils were pre-starved for 12 hours. In the first experiment, susceptible host-plant material was placed in one tube, resistant material in the opposite tube, and the other two tubes were left blank. The second experiment consisted of susceptible host-plant in one tube and the other three with resistant material. The third experiment consisted of susceptible host-plant in one tube and the other three blank. For each experiment twenty weevils were introduced into the centre of the olfactometer chamber and counted every 10 minutes for one hour. All experiments were performed at night using a "Varo Noctron V" night-scope for making observations. The olfactometer was rotated several times throughout the duration of the experiment to minimise any directional bias. After each experiment, the apparatus was dismantled, thoroughly washed with hot detergent, swabbed with 90% ethanol and rinsed with distilled water several times. It was then dried in an oven at 60°C for two hours.

Natural Volatiles Analysis.

Oxygen-free nitrogen gas was bubbled through a container of distilled water and then through a chamber which contained an intact ryegrass plant at a rate of 44 ml/min for four to five hours (calculated with a 10 ml bubble gauge flowmeter). The naturally emitted volatile chemicals from the ryegrass were trapped on solid adsorbent Tenax GC (60/80). Trapped volatiles were washed off with diethyl ether and transferred directly onto a 50 m Carbowax 20M glass SCOT GC column and analysed by gas chromatography (GC) and mass spectroscopy (MS). Peaks were identified either by Kovats Indices (reference to a series of hydrocarbons) and compared to published values of identified volatiles (Jennings and

Shibamoto 1980), or from a "Shimazdu" QP1000 GCMS trace. Both the susceptible (no endophyte) and resistant (endophyte-positive) ryegrasses were each tested **once**. Cut ryegrasses were not tested because of inherent difficulties in identifying the small amounts of compounds emitted from cut surfaces, and the possible release of additional volatiles from the wounded surface.

Orientation after Antennectomy.

All antennectomies were performed after the weevil had been anaesthetised with CO_2 gas. An initial experiment involved comparing the orientational responses to a food source of adult weevils which had been anaesthetised with CO_2, with those not subject to CO_2. This was done in petri dishes and involved testing the firmly established feeding preference behaviour of ASW with respect to endophyte-infected and endophyte-free ryegrasses, which is more specific than a host/non-host relationship (Barker *et al.* 1984c; Gaynor *et al.* 1983, and Chapter 4 of this thesis). This comparison involved placing a 3 cm section of ryegrass leaf (Grasslands Nui perennial ryegrass), cut at the ligule from an endophyte-infected plant, next to an identically cut section from an endophyte-free clonal replicate in a petri dish which contained a piece of water-moistened Whatman No. 1 filter paper. A single adult weevil was introduced into this arena for each comparison and left for 48 hours at 16°C with a 16: 8 hour photoperiod. Twenty replicates were performed for each test. During a pre-test starvation period of 24 hours weevils had no access to water or food. Upon completion, each leaf was scored for feeding damage on a scale of 0 to 3, (0 - having no feeding holes; 1 - one feeding hole; 2 - two to three feeding holes, and 3 - greater than three feeding holes, Gaynor *et al.* 1983). The significance of the differences in feeding scores between the two leaf sections were tested using the Wilcoxon signed rank test (Siegal 1956).

Further tests were conducted to compare "normal" intact weevil orientational responses (with respect to feeding between susceptible and resistant host plants), with those with antennae or parts thereof removed as follows: a) both antennae completely removed, b) one antenna completely removed, and c) clubs only of both antennae removed. The antennectomies were performed, after anaesthetising the weevils under CO_2 gas, by cutting off the antennae with opticians surgical scissors. In addition, 20 weevils with their antennae removed were introduced into the olfactometer where two tubes contained the susceptible ryegrass and two tubes were left blank to compare the orientational responses of these weevils.

RESULTS.

Collection of ASW Adults.

Adequate numbers of weevils were usually collected for the ensuing experiments under conditions of wind speeds less than 25 km/hr and temperatures above 14°C. In cooler conditions slugs and dew caused the weevils to accrete into slimy boluses, paralleling Goldson's (1979a) problems. Furthermore, feeding intensity was reduced in cooler conditions because the weevils remained inactive in plant crowns and under debris on the soil surface, and this also resulted in lower catches.

Visual Orientation.

The spectral compositions of each colour, as determined by the spectrophotometer, are as follows: red - has a peak reflectance curve between 600 - 700 nm and a smaller reflectance between 350 - 540 nm. Green peaked at 510 nm, yellow at 500 - 700 nm, and blue at 380 - 540 nm. The results of feeding choices between colours show no significant differences between any of the colour combinations offered (Table 2). These weevils did not visually discriminate between any of the colour combinations, choosing to feed on each coloured plug equally. Also, during the 16 hour light phase when constant observations were made upon selected weevils, no indication of flight behaviour was evident.

Olfactory Orientation.

No significant results were obtained using the olfactometer. Weevils moved into each arm of the olfactometer randomly, irrespective of combinations of plant material and blank tubes (Table 3). (X^2 = 1.44, d.f. = 11, N.S.).

Natural Volatiles Analysis.

Compounds identified from the head space over the ryegrasses are listed in Table 4. Differences between the susceptible and resistant ryegrass cultivars are slight, reflecting amounts of compounds emitted rather than specific compounds, presumably as a result of the differing times the two ryegrasses were held in the collection chamber.

Table 2.

Visual Orientation of Adult Argentine Stem Weevil to Various Coloured Nutrient Agar Plugs.

Treatment		% Weevils Showing a Feeding Response			
A	B	A Preferred	B Preferred	No Preference	No Feeding
Red	Red	20 ns	10	60	10
Red	Green	30 ns	20	50	0
Red	Blue	20 ns	40	40	0
Red	Yellow	20 ns	20	60	0
Red	White	20 ns	30	50	0
Green	Red	20 ns	30	50	0
Green	Green	30 ns	30	40	0
Green	Blue	30 ns	20	40	10
Green	Yellow	30 ns	30	40	0
Green	White	20 ns	10	70	0
Blue	Red	30 ns	30	40	0
Blue	Green	30 ns	20	40	10
Blue	Blue	20 ns	20	60	0
Blue	Yellow	20 ns	30	50	0
Blue	White	20 ns	20	50	10
Yellow	Red	30 ns	20	50	0
Yellow	Green	30 ns	30	40	0
Yellow	Blue	30 ns	20	40	10
Yellow	Yellow	30 ns	20	50	0
Yellow	White	20 ns	20	60	0
White	Red	30 ns	20	50	0
White	Green	20 ns	10	60	10
White	Blue	30 ns	20	40	10
White	Yellow	20 ns	10	70	0
White	White	10 ns	20	60	10

ns = Not Significant (Wilcoxon signed-rank test (Siegal 1956)).

Table 3

Weevil Response to Odour from Four Sources in the Olfactometer.

	Number of Weevils Entering Each Olfactometer Arm (sum of five replicates)			
Arm	A	B	C	D
Experiment 1	Susceptible Ryegrass 26	Resistant Ryegrass 23	Blank 27	Blank 24
Experiment 2	Susceptible Ryegrass 27	Resistant Ryegrass 25	Resistant Ryegrass 27	Resistant Ryegrass 21
Experiment 3	Susceptible Ryegrass 25	Blank 25	Blank 26	Blank 24
Antennectomised Weevils (one test with 20 weevils)	Susceptible Ryegrass 6	Susceptible Ryegrass 5	Blank 2	Blank 5 *

* Two weevils failed to make a choice and remained within the centre of the chamber.

Table 4.

Major Volatile Compounds Emitted from the Head Space Over a Susceptible and Resistant Ryegrass Cultivar, as Identified by Gas Chromatography and Mass Spectroscopy.

1 Bicyclo 7.2.0 undec-4-ene, 4,11,11-trimethyl-8-methylene IR-(IR*,4E,9S*)-(9CI)
MW 204 (Caryophyllene) #

2 Octanoic Acid
MW 144

3 3-Hexen-1-ol, acetate
MW 144

4 2H-pyran-2-one, tetrahydro-6-propyl-(8CI9CI)
MW 142 (δ Octalectone)

5 Hexadecanoic acid
MW 256

6 Bicyclo 3.1.1 Hept-2-ene, 2,6,6-trimethyl-(9CI)
MW 136 (α-pinene) #

7 Cyclohexene, 1-methl-4-(1-methylethenyl)-(9CI)
MW 136 (Limonene)

- closest possible match

Orientation after Antennectomy.

The initial experiment of anaesthetising the weevils with CO_2 gas had no adverse effect on weevil feeding or discrimination between susceptible (endophyte-free) and resistant (endophyte-infected) host-plant material ($P < 0.001$ Table 5). Also, antennectomised weevils could discriminate between the susceptible and resistant ryegrass tissue, irrespective of treatment ($P < 0.001$ Table 5). Removal of one or both antennae, or both clubs (i.e. the distal four segments of the antennae) produced a small decline in feeding on ryegrasses in petri dishes, with the lowest level of feeding observed when both antennae were completely removed or when both clubs were removed. However, in all cases weevils could still discriminate between endophyte-infected and endophyte-free ryegrasses. The feeding differences can be accounted for by a higher percentage of antennectomised weevils failing to feed rather than failing to discriminate. These results are also summarised in Table 5.

The results of the olfactometer test with antennectomised weevils were also not significant, with equal numbers of weevils entering each of the four arms (Table 3).

DISCUSSION.

From the test results it appears that colour discrimination is not important in the host-plant selection process for ASW (Table 2). Whether this is an artefact produced within the laboratory under the conditions imposed by the experiment, such as the restricted environment of a petri dish, or an internal physiological state of the insect, such as satiation from the adequate and readily available food source, is not known. There are many differences between laboratory conditions and natural field conditions, it is thus difficult to extrapolate and interpret results adequately. Undoubtedly, the internal physiological state of an insect is very important, but cannot be positively determined. For example, it is known that certain physiological changes in aphids render them more responsive to short wavelengths of light from the sky, and they initially fly upwards. Later, the phototactic response is switched, and they react positively to the lower wavelength light from vegetation, and so land (Moericke 1955), but the "physiological changes" themselves remain obscure. It may thus be that the weevils tested in these experiments were in the "wrong" physiological state to visually discriminate between the colours.

Table 5

The Orientational Responses of Argentine Stem Weevil with Respect to Food Choice Before and After Various Ablation Treatments.

Treatment	% Weevils Showing a Feeding Response				No. of weevil's tested
	Susceptible host preferred	Resistant host preferred	No preference	No feeding	
CO_2 anaesthetisation	80 **	10	0	10	20
No anaesthetisation	90 **	10	0	0	20
No antennae removed	90 **	0	0	10	40
1 antenna removed	85 **	5	0	10	20
2 antennae removed a)	60 **	7	0	33	30
2 antennae removed b)	65 **	0	0	35	20

** = $P < 0.001$. (Wilcoxon signed rank test).

a) = both antennae completely removed

b) = both antennal clubs removed

Possibly the weevil needs to be in flight to discern colours, but flight is impossible within the confines of a petri dish. Even so, no flight initiating behaviour was evident when weevils were observed within the petri dishes over 16 hours. In the field, however, **mass** flight behaviour has been recorded in the South Island of New Zealand, but not in the North Island (Pottinger 1961a, 1966; Barker and Pottinger 1981; and Goldson 1981a). Up to 1,299 adult weevils were caught on sticky traps in the South Island compared to only 44 in the North Island (even though there were high numbers of weevils in both pastures - 1,000 / m^2) (Prestidge *et al.* 1983). With respect to visual discrimination of colours during dispersive flight periods, Pottinger (1966) found that weevils were caught in red, green, white, or yellow traps, with yellow coloured traps being the most effective. In observed flights it was noticed that weevils alighted on the traps and were not passively blown onto them, although when wind speeds exceed 4.5 km/hour ASW drift with the breeze as they are clumsy fliers unable to make strong or active flight (Pottinger 1966). Similar flight behaviour has been recorded for other weevils (Prokopy and Gyrisco 1965; and Blickenstaff *et al.* 1972).

Pottinger's (1966) work therefore indicates that weevils can adequately discriminate between colours during flight. Possibly the South Island population, which has a high overwintering survival rate in comparison to the poor survival rate in the North Island, greatly reduces the availability of suitable oviposition and larval development sites for the second generation, and hence dispersal flights are essential to reach new feeding and oviposition sites for continued survival (Pottinger 1961a). It is likely that at such times the visual discrimination of colours is important in the host-plant selection process for ASW.

Experiments with the olfactometer also proved negative with both intact weevils and those with antennae removed (Table 3). The olfactory sense does not appear to be involved to any great extent in ryegrass host-plant perception from distances up to 15 cm. Weevils moved into each arm of the olfactometer in equal numbers, irrespective of the odour source. The naturally emitted odours themselves, largely consisting of 3-hexene-1-ol acetate, are widely distributed in plants, including pasture species (Visser 1979; Visser and Ave 1978; Visser *et al.* 1979; Buttery *et al.* 1982, 1984; Hopkins *et al.* 1985; and Rowan pers. comm. 1987). For ASW these compounds alone seem unlikely to offer suitable cues for the insect to discriminate between potential food plants, but rather just a general indication of the presence of green leaves. This is consistent with the results obtained from within the olfactometer between the susceptible and resistant host plants, but does not explain the lack of orientational response between the "food" and blank tubes, especially considering that the weevils were starved for 24 hours prior to the experiment. However, the equal numbers of weevils in each arm of the olfactometer may indicate an over-riding preference for humidity or water, as the test weevils had no access to water during the pre-test starvation period. Humidity has been implicated in the host-plant selection process of the alfalfa weevil, which possess extremely sensitive hygroreceptors. Adult alfalfa weevils can distinguish humidity differences as small as 5% (Springer and Pienkowski 1969). While water vapour alone is probably not sufficient to lead weevils unerringly to host plants, it seems likely that the humidity gradient immediately surrounding a plant could be one link in a chain of orientation stimuli. Humidity preferences were not tested in the olfactometer experiments but kept constant throughout. It seems possible, however, that humidity would only enable discrimination between host plants at relatively short distances and thus cannot be implicated in the host-plant selection process from a distance.

While negative in the tests, the volatiles analysis and suppositions concerning the role of humidity suggest that these parameters should be further investigated. Furthermore, the chemicals identified by gas chromatography and mass spectroscopy could be purified and reintroduced at various concentrations to ASW's in separate olfactory bioassays. In addition, if specific receptors can be identified (through scanning electron microscopy (SEM)), and classified (through transmission electron microscopy (TEM)), electrophysiological recordings from specific olfactory receptors could be undertaken with respect to specific individual chemical compounds, and thus very precise information on olfaction obtained. Chapter 6 provides the beginnings for such research.

Negative results were also obtained from the orientation tests in the petri dishes. Beetles rendered anosmic by antennectomy were inferior to "normals" in feeding intensity but not in host location ability. Weevils could discriminate between endophyte-infected and endophyte-free material in all tests. The lowest level of feeding observed was when both antennae were removed, or when both clubs were removed. This reduction in feeding may be assumed to be the direct result of loss of olfactory receptors, although careful observations of the leaf tissue after conclusion of the experiments revealed "test" bites on many of the excised leaves of the endophyte-positive plant tissue. This indicates that plants may not be rejected on the basis of olfaction alone, but more likely that tactile and/or gustatory senses are also important in host-plant selection by ASW.

With respect to other weevils, Pienkowski and Golik (1969) measured the orientation responses of adult alfalfa weevils (*Hypera postica* (Gyllenhal)) to the odour of alfalfa, and found that the weevils had a directed food-perception distance of only 3 to 5 mm. If such small distances are applicable to ASW, then orientation to potential host-plants from a distance cannot be of an olfactory nature. Again, however, it must be emphasised that the necessary physiological state of the insect may be important. Thorsteinson (1960) for example states that the sweet clover weevil (*Sitona cylindricollis* (Fahraeus)) is responsive to plant odour only on the wing during its dispersal flights.

Conflicting evidence has accrued with respect to both colour and olfactory orientation of weevils. Mitchell and Taft (1966), Keller *et al.* (1963), and Neff and Vanderzant (1963) believe odour is important for the boll weevil (*Anthonomus grandis*). In contrast, Merkl and Mayer (1966), Hollingsworth *et al.* (1964) and Taft *et al.* (1969) agree that visual discrimination occurs between colours. However, Mitlin *et al.* (1966) and Stephens and Lee (1961), state that colour is not important. Similarly, it is believed that the alfalfa weevil is able to locate alfalfa for feeding by responding to visual cues (Meyer 1975, 1977; Meyer and Raffensperger 1974c). Yet humidity gradients surrounding the plant and humidified volatiles released by alfalfa also may produce positive responses (Byrne and Steinhauer 1966; Byrne *et al.* 1966 and Meyer and Raffensperger 1974a and b). These olfactory cues seem to predominate only at very short distances of perhaps 5 mm or less (Pienkowski and Golik 1969). Byrne and Steinhauer (1966) reported that removing the antennal club from either of the weevil's antennae reduced feeding by the insect as well as its ability to orient toward the alfalfa. They attributed this reduction to a loss of olfactory sites on the club. Bland (1981) stated that alfalfa volatiles play a subordinate role to moisture as an attractant until weevils are within a few mm, and olfactory responses to moisture predominate over visual cues when the attractant was less than 40 mm away.

This conflicting evidence may simply be accounted for by the insect's physiological state and restraints imposed by the equipment. The physiological state of an insect is a variable which continually crops up, offering a plausible explanation to many unanswered questions and contradictory evidence.

Perhaps the most plausible explanation for host-plant location by ASW at the present time with the limited information available is one of chance, or rather, of "apparency" (Feeny 1976), i.e. plant species which are readily discovered by grazers. Agriculture creates highly concentrated, artificial patches of plants. In New Zealand, perennial ryegrasses alone cover 7 million hectares (Siegal *et al.* 1985) and is thus a highly apparent species. So there is a very high probability that a flying weevil will select a suitable host-plant simply if it lands on something green. Once established on a suitable host-plant further dispersal may not be necessary, as the entire life cycle of ASW can be completed on ryegrasses. Lance (1982) adequately sums up - "For generalist herbivores which feed on highly apparent plants, random contact with foliage often will result in contact with acceptable food. Thus host-plant location in many generalists does not require accurate, specific, long-distance orientation mechanisms. However, the host-plants of generalists do vary in their suitability, preferentially feeding on the most suitable plants in a locale." The next chapter will explore the effects of feeding by ASW on ryegrasses.

CHAPTER 4

FEEDING.

INTRODUCTION.

Damage to improved pastures throughout New Zealand is caused by the feeding activities of both adult and larval ASW. There is, however, evidence that pasture grasses vary in their susceptibility to stem weevil feeding (Kelsey 1958; Whatman 1959; Hoy 1960; Pottinger 1961a and b; Timlin 1964; Goldson 1979 and Gaynor and Hunt 1983). It is suggested that this susceptibility is related to all three recognised mechanisms of plant resistance:- non-preference, antibiosis and tolerance. Tolerance is influenced by water and temperature relationships of the plant, and by cropping and grazing management. For example, in dry summers annual ryegrasses may be unable to tiller and grow satisfactorily in the face of ASW attack, with the result that tiller death of up to 98% has been recorded (Kelsey 1958). Non-preference, on the other hand, is imparted either by the physical nature of the plant surface or by deterrent biochemical factors. Hairy leaf sheaths are thought to be the main reason for low susceptibility of Yorkshire fog, paspalum, and prairie grass to ASW, while biochemically, ryegrasses infected with the endophytic fungus *Acremonium lolii*, Latch, Christensen and Samuels, (Latch *et al.* 1984), have been found to be resistant to adult and larval feeding and adult egg laying because of deterrent compounds (Gaynor *et al.* 1983; Barker *et al.* 1985a). These same compounds have also been implicated in the antibiosis form of resistance (Barker *et al.* 1984a and b). Prior to the discovery of ASW resistance being linked to the presence of *A. lolii* endophyte (Prestidge *et al.* 1982; Mortimer *et al.* 1982) considerable variability and contradictory evidence was apparent in results of studies on ASW interactions with various ryegrass cultivars (Pottinger 1961a; Kain *et al.* 1977, 1982; Goldson 1979a, 1982; Barker and Pottinger 1981; Barker *et al.* 1981; and Gaynor and Hunt 1983). The percentage of plants infected with endophyte can now explain many of these earlier cultivar anomalies, but differences in feeding preferences may still exist when ryegrass cultivars have no endophyte within their tissue (Gaynor and Hunt 1982, 1983). Gaynor and Hunt (1983) called such factors genotypic, but did not attempt to investigate them.

As ryegrasses are the preferred host plants of ASW (Prestidge *et al.* 1985c) a selection of all available ryegrass cultivars was examined for feeding preferences by adult ASW. The endophytic fungal association is very important, therefore all cultivars were examined for endophyte presence, and the levels recorded as ELISA indices (Musgrave 1984). Also, as weevils feed selectively on specific parts of ryegrass plants (Kelsey 1958), and endophyte distribution is known to be confined to specific areas (Musgrave and Fletcher 1984), endophyte distribution within these plants was also determined. Furthermore, to test whether adult ASW's were responding to an extractable material within infected ryegrass, and to confirm the biochemical relationships established by Gaynor *et al.* (1983), Rowan and Gaynor (1986) and Gaynor and Rowan (1985), extracts from clonal replicates of ryegrass infected and uninfected with endophyte were prepared. Also, in an attempt to define the "genotypic" factors mentioned by Gaynor and Hunt (1983), each cultivar was analysed for leaf strength and fibre content, as inter-specific leaf strength differences exist between ryegrasses which are associated with palatability to grazing animals (Beaumont *et al.* 1933 and Rae *et al.* 1964). These differences were later related to fibre content by Archibald *et al.* (1943), Kneebone (1960), and Bailey (1964). Farm animals prefer species with lower fibre content and strength in the order annual > hybrid > perennial (Evans 1964). Several authors have also stated that this trend of annual > hybrid > perennial follows for ASW feeding (Kelsey 1958; Pottinger 1961a and b; Goldson 1979; and Gaynor and Hunt 1982). In addition, as the above plant factors must interact with the insect, variable features of the weevil were examined. These were age, size, and sex, plus the general behaviour with respect to feeding. Finally, general morphological differences between cultivars were examined.

MATERIALS and METHODS.

All weevils were field collected 48 hours prior to commencing experiments and held in containers supplied with water but no food.

Feeding Preference Tests.

Cultivars used in this experiment are listed in Table 6. Each cultivar was grown from seed sown in trays in early June 1984. A mixture of equal parts sand, perlite and peat was used, with added lime, superphosphate, and "Osmocote" slow release fertiliser (N : 14, P : 6, K : 11). The plants were raised in a glasshouse until mid August with natural light and temperature, and watered twice daily by capillary bed. They were trimmed regularly with scissors to maintain good vegetative growth. To test feeding responses to an individual ryegrass cultivar, adult weevils were individually given a choice between a 3 cm section of a leaf cut at the ligule from endophyte-free Nui perennial ryegrass, compared with an identical section of leaf cut from the test cultivar. Both leaf portions were placed in a petri dish on water-moistened Whatman number 1 filter paper. There were sixty replicates of each test. The experiments were started on September 5th and maintained at 16°C with a LD 16: 8 hour photoperiod for 48 hours. Upon completion, each leaf portion was scored for feeding damage on a scale of 0 to 3, (0 - having no feeding holes; 1 - one feeding hole; 2 - two to three feeding holes; and 3 - more than three feeding holes). The significance of differences in feeding scores between the two leaf sections was tested using the Wilcoxon signed rank test (Siegal 1956).

Endophyte Incidence.

Twenty tillers from various plants of each ryegrass cultivar were cut into three regions: the tip of the leaf lamina, the basal section of the leaf lamina above the ligule, and the leaf sheath region. Each region from every cultivar (Table 6), was tested using the enzyme-linked immunosorbent assay (ELISA) of Clark and Adams (1977), to assess the quantity of endophyte present (ELISA index). Sample preparation and detection followed the method of Musgrave (1984). Sampling coincided with the feeding preference trial above.

Microscopic examination of tissue was also carried out to confirm the presence of endophyte. The procedure involved removing a leaf with its sheath from the plant, cutting the adaxial epidermis with a scalpel and mounting a strip of this epidermis on a microscope slide in a solution of lactophenol which contained analine blue. The slide was warmed for a few seconds over a flame to accelerate staining of mycelium.

Table 6

Ryegrass Cultivars Evaluated in Adult Argentine Stem Weevil Feeding Tests.

Cultivar Name	Botanical Name	Endophyte (+ present, - absent)	Name Used in Text
"Grasslands Tama"	*Lolium multiflorum* Lam.	-	Tama
"Grasslands Moata"	*L. multiflorum* Lam.	-	Moata
"Grasslands Paroa"	*L. multiflorum* Lam.	-	Paroa
"Grasslands Manawa"	*L.* (*multiflorum* X *perenne*)	-	Manawa
"Grasslands Ariki"	*L.* [(*multiflorum* X *perenne*) X *perenne*]	-	Ariki -
"Grasslands Ariki"	*L.* [(*multiflorum* X *perenne*) X *perenne*]	+	Ariki +
"Grasslands Ruanui"	*L. perenne* L.	-	Ruanui -
"Grasslands Ruanui"	*L. perenne* L.	+	Ruanui +
"Grasslands Nui"	*L. perenne* L.	-	Nui -
"Grasslands Nui"	*L. perenne* L.	+	Nui +
Yates "Ellett"	*L. perenne* L.	-	Ellett -
Yates "Ellett"	*L. perenne* L.	+	Ellett +

Effect of Leaf Region on Feeding Preference.

Earlier attempts to determine feeding preferences used leaf tips (e.g., Gaynor *et al.* 1983; Kain *et al.* 1982). As endophyte levels vary with the plant tissue, (i.e., sheath, leaf base, and leaf tip, (see above and later results)), it is important to test these tissues separately. Thus leaf tips and leaf bases were compared for feeding preferences using both endophyte-free and endophyte-positive plants. Tests were scored for feeding damage on a scale of 0 to 3 (as above), and analysed using the Wilcoxon signed rank test (Siegal 1956).

Effects of Endophyte on Feeding.

To minimise plant genotypic effects and thus to determine the effect of endophyte alone on ASW feeding, Grasslands "Nui" perennial ryegrass infected with *Acremonium lolii* endophyte was compared with endophyte-free clonal replicates in a feeding trial. Clonal Nui plants without endophyte were produced according to the method of Latch and Christensen (1982), using a Benomyl fungicide (Benlate 0.1 kg a.i. / litre of potting mix) treatment. The plants were repotted several months prior to experimentation, and checked microscopically for infection. One infected leaf and one uninfected leaf was presented to each of 60 weevils held individually in petri dishes in mid October 1985. The amount of feeding after 48 hours was scored on the 0 to 3 scale previously described. Differences in feeding scores between the infected and uninfected leaves were calculated and the significance of the deviation of these differences from zero were determined using the Wilcoxon signed rank test (Siegal 1956).

Effect of Extracts of Ryegrass Leaf Sheaths on Feeding.

To determine the effect of various plant extracts on adult weevil preference, two 1 cm diameter agar discs containing 4% Davis agar, 4% cellulose powder and 5% sucrose were presented to one adult weevil in a petri dish. One disc contained a known amount of crude extract from endophyte-infected ryegrass adsorbed onto cellulose powder, while the other disc contained the same amount of extract from endophyte-free clonal replicates. The final concentration of extract in the agar was 2.5% which corresponds to concentrations of extractable material in the plant tissue. The extract was obtained by blending the sheath region of a test plant in 95% ethanol. This was filtered through a sintered glass funnel containing Celite 545 and adsorbed to the cellulose powder in a "Buchi" rotary evaporator at

40°C. Forty weevils were used in each of three separate tests run at 16°C with a LD 16: 8 hour photoperiod for 72 hours. The amount of feeding on each disc was scored on the 0 - 3 scale (as above), and feeding scores analysed using the Wilcoxon signed rank test (Siegal 1956). Tests were conducted in late January 1985.

To compare the nature of the present extract with that obtained by Rowan and Gaynor (1986) and Gaynor and Rowan (1985), the 95% ethanol extracts obtained above were solvent partitioned into two phases, a chloroform - methanol phase (CM) and a methanol - water phase (MW). The CM phase was further partitioned between petroleum ether and 80% - ethanol, and the MW phase partitioned to n-Butanol and water. The extract was then dialysed against three changes of 10% ethanol and water through "Gallenkamp" dialysis tubing, which has a molecular weight cutoff between 12,000 and 14,000. Further partitioning was unnecessary as the preferences exhibited by the weevils at this stage for either disc would confirm the biochemical nature of the extracts when compared with the complete partitioning performed by Rowan and Gaynor (1986). Extracts from both endophyte-infected and uninfected ryegrasses were partitioned and compared in the choice bioassay at concentrations proportional to that in the original extract. Extracts were considered to be "active" if, in the choice bioassay, weevil feeding was significantly less on the disc containing extracts from infected ryegrass.

Leaf Strength.

The methods followed were those of Evans (1964 and 1967b). Samples of all ryegrasses (Table 6) were tested for leaf strength between 18 and 29 March 1984. Fifty leaves were taken at random from each cultivar, and tested by a longitudinal pull (i.e. only tensile strength was measured), using the apparatus described by Evans (1967a). This breaking load was measured on a 5 cm length of leaf cut just above the ligule. After being broken, the specimens were oven dried at 105°C overnight, and then weighed. Leaf strength is here defined as: breaking load (g) ÷ dry wt (mg) of 5 cm of leaf.

Fibre Content.

Ten grams of fresh ryegrass leaf lamina tissue from each cultivar in Table 6 were freeze-dried for 24 hours, then finely ground in a mortar and pestle. One gram of this sample was then analysed for total fibre content by the neutral-detergent fibre determination method described by Robertson and Van Soest (1963).

Effects of Adult Size, Sex and Age on Feeding.

Obvious differences in size existed within the field-collected weevils. A test was therefore conducted to determine the effect of size and sex on feeding. One hundred each of the largest and smallest weevils were selected. The two groups were sexed and subdivided on the basis of size into two further groups to give large males, small males, large females, and small females. Females were distinguished from males by the shallow depression in the 7th sternite, compared to the two projections on the 7th sternite of males. Similar, but less obvious, is the slight bulge in the anterior median part of the females 3rd abdominal sternite compared to that of the male which is concave (Goldson 1979a). Size is not a good indication of sex, though females tend to be larger than males, with a mean length of 2.1 mm compared to 1.9 mm for males, but females have been found to fall within the male range of size. The experiment consisted of placing one 3-cm section of leaf blade cut from the ligule of a "Nui -" perennial ryegrass plant into each of 40 petri dishes which contained moistened filter paper. Ten large male weevils were placed separately into 10 petri dishes, as were 10 large females, 10 small males and 10 small females. Tests were conducted in mid March 1985. Weevil feeding scars were counted and scored (0 - 10 scale) after 96 hours in darkness at 16°C. For the feeding scores, 0 represented no feeding and 10 the maximum feeding for the bioassay, on the basis of leaf area consumed (Barker *et al.* 1984b).

Koehler and Gyrisco (1963) and Barnes and Ratcliffe (1967) reported that feeding of newly emerged alfalfa weevil adults decreased with time. After one evening of sweep netting for adult ASW weevils, numerous teneral adults were caught, recognisable by their colour difference from older weevils; light red in comparison to a dull grey (Pottinger 1961a). A short test was therefore conducted to determine if ASW exhibits the same pattern as alfalfa weevil. One of these newly emerged adults (less than 7 days old (Pottinger 1961a)) and one older weevil (more than 40 days old - maintained in the laboratory on an agar based diet after being used in earlier feeding tests), were placed into separate petri dishes which contained a 3-cm section of "Nui -" ryegrass leaf and moistened filter paper. The test was replicated twenty times. The amount of feeding on each leaf blade was scored visually on a scale of 0 to 10 (as above) after 96 hours in darkness at 16°C and compared between each age class.

Behavioural Observations.

Visual observations were made of weevils in a no choice situation and a choice situation when given an endophyte-infected and endophyte-free leaf base from clonal replicates of Nui ryegrass. Observations were continuous for two hours in four separate trials. Weevils were food-starved for 24 hours prior to observations.

RESULTS.

Feeding Preference Tests.

Table 7 gives the results of the feeding preference tests of adult ASW when offered a choice of ryegrass leaves from two different cultivars. Figure 2 shows the same data graphically, where the results have been normalised in comparison with Nui - (which was set at 50). By standardising the feeding responses to one plant, preferences can be more readily compared quantitatively. From these results it is evident that ASW preferred to feed most on the annual ryegrasses (Tama, Moata, and Paroa) and the hybrids (Manawa and Ariki -). Endophyte-free perennials (Ruanui -, Nui -, and Ellett -) were preferred next, and endophyte-infected cultivars (Ariki +, Ruanui +, Nui +, and Ellett +) preferred least.

Endophyte Incidence.

The mycelium of *A. lolii* endophyte appears under the light microscope as septate sparsely branched intercellular hyphae running longitudinally in the leaf sheath (Plate 1). The relative amounts of endophyte present in each plant, expressed as ELISA indices (Table 8), varied considerably within cultivars depending upon the region of sampling. Leaf sheaths contained the most endophyte, with values ranging from 0.871 for Tama to 7.324 for Ellett +.

Basal leaf lamina tissue, which corresponds to the tissue used in the feeding preference trial, also contained appreciable amounts of endophyte. ELISA values ranged from 0.769 for Tama to 3.615 for Ellett + (Fig. 3). Endophyte levels in leaf tip regions were low and ranged from 0.818 to 1.469. Only Nui + and Ellett + contained endophyte in significant amounts in this region. According to Musgrave (1984) ELISA indices below 1.1 indicate a 99% probability of there being no endophyte present, hence all annual and endophyte-minus cultivars listed in Table 6 can be considered endophyte free.

Table 7

Feeding Preferences of Adult Argentine Stem Weevil When Offered a Choice of Leaves from Two Ryegrasses.

% Weevils Showing a Feeding Response

Ryegrass A		Cultivars B	Leaf A preferred	Leaf B preferred	No preference	No feeding
Nui -	:	Tama	12	83 **	3	2
Nui -	:	Moata	13	75 **	7	5
Nui -	:	Paroa	17	67 **	8	8
Nui -	:	Manawa	15	75 **	2	8
Nui -	:	Ariki +	61 **	20	17	2
Nui -	:	Ariki -	30 ns	22	41	7
Nui -	:	Ruanui +	53 **	22	12	13
Nui -	:	Ruanui -	27 ns	17	51	5
Nui -	:	Nui +	91 **	7	2	0
Nui -	:	Nui -	17 ns	18	62	3
Nui -	:	Ellett +	84 **	8	5	3
Nui -	:	Ellett -	30 *	17	45	8

Sixty weevils were used in each test.

Significant preferences were determined by the Wilcoxon signed rank test (Siegal 1956).

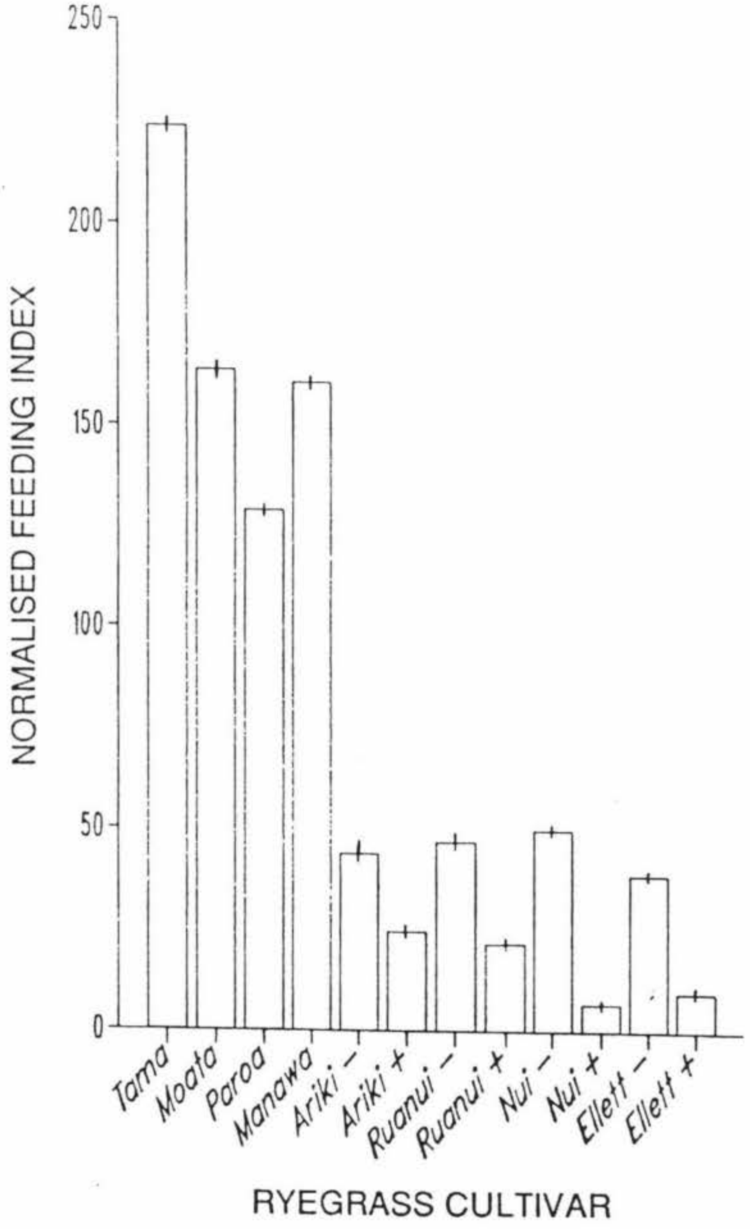
ns = not significant,

* = P < 0.01,

** = P < 0.001

Figure 2

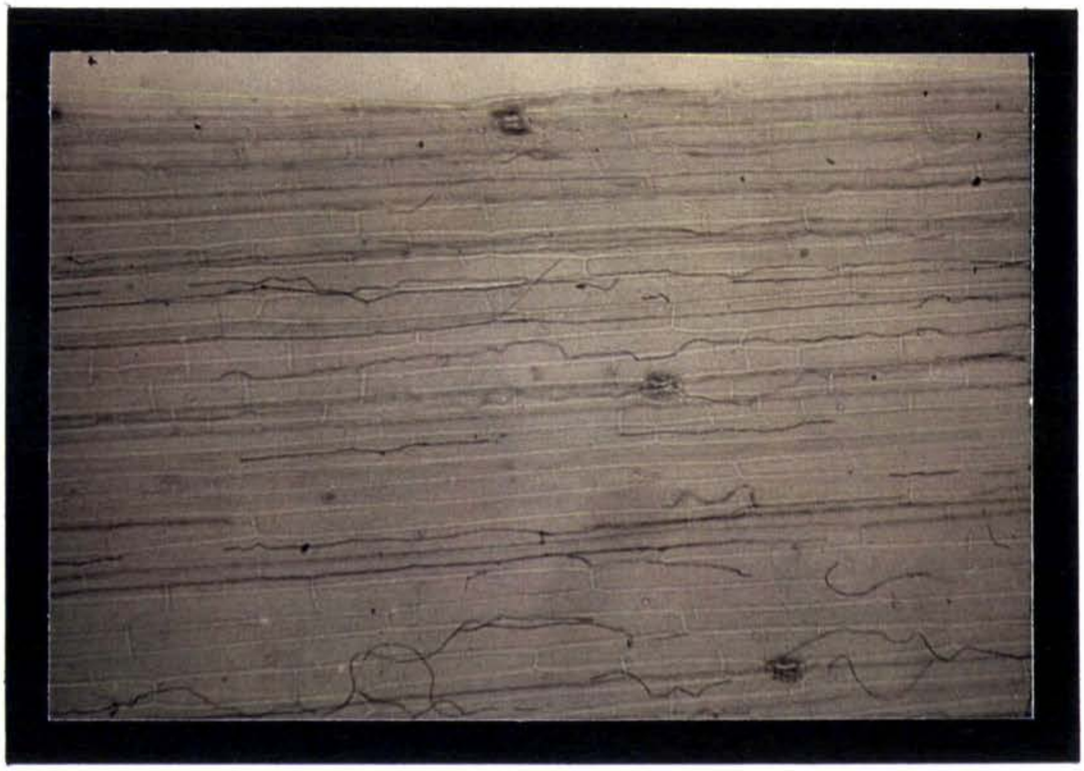
Relative Feeding Preferences of Argentine Stem Weevil for Various Ryegrass Cultivars (normalised to Nui -).



Columns represent mean feeding scores for 60 weevils.

Standard Errors are indicated by vertical lines on the columns.

Plate 1



The mycelium of *Acremonium lolii* endophyte in the leaf sheath region of a Nui ryegrass plant, as viewed under the light microscope. (Magnification X 400).

(photo by G.C.M. Latch)

Table 8

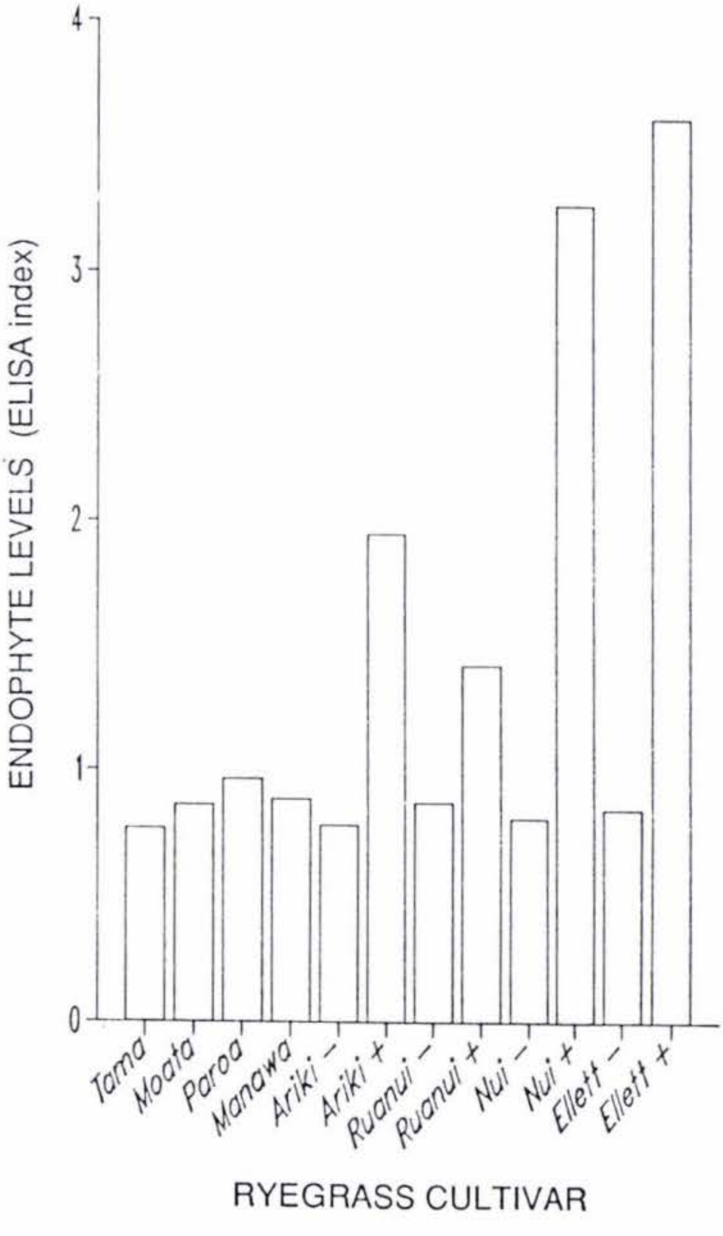
Endophyte Levels from Three Regions of Each of the Ryegrass Cultivars. *

Cultivar	Endophyte Incidence (ELISA Indices).		
	Tiller Region		
	leaf sheath	leaf base	leaf tip
Tama	0.871	0.769	0.818
Moata	0.957	0.861	0.910
Paroa	1.086	0.962	0.889
Manawa	1.071	0.885	0.856
Ariki +	5.754	1.948	1.141
Ariki -	0.886	0.782	0.862
Ruanui +	5.114	1.423	0.933
Ruanui -	0.971	0.872	0.819
Nui +	6.514	3.269	1.343
Nui -	0.886	0.808	0.867
Ellett +	7.324	3.615	1.469
Ellett -	0.903	0.864	0.891

* as at 5th September 1984.

Figure 3

Endophyte Levels from the Base of Leaf Lamina Tissue of Various Ryegrass Cultivars.



Effect of Leaf Region on Feeding Preference.

There was no significant feeding preference by weevils for leaf tips of Ariki ryegrass with or without endophyte. When using Nui ryegrass **tips** with or without endophyte, however, there was a significant preference for the endophyte-free samples ($P < 0.01$). There was an even more significant result with endophyte-infected and endophyte-free leaf base tissue from Nui, with the Nui-minus tissue again being most preferred (Wilcoxon signed rank test, $P < 0.001$) (Table 9).

Effects of Endophyte on Feeding.

Eighty-three percent of weevils responded by feeding on an endophyte-free Nui leaf, no weevils preferred the endophyte-positive leaf, 7% showed no preference and 10% did not feed. Thus the differences between *A. lolii*-infected leaves and uninfected clonal replicates was highly significant (Wilcoxon signed rank test, $P < 0.001$).

Plate 2 shows an example of such a petri dish feeding bioassay after 12 hours.

Effects of Extracts of Ryegrass Leaf Sheaths on Feeding.

The results of tests in which crude 95% ethanol extracts were incorporated into agar discs and presented to weevils in a choice bioassay are presented in Table 10. Weevils fed more on the discs incorporating extracts from uninfected plants ($P < 0.01$). The scheme used for partitioning and dialysis of solvent extracts and results of bioassays are shown in Figure 4. Activity was concentrated mainly in the more polar phases (i.e. n-Butanol and water).

Plate 3 shows an example of a petri dish feeding bioassay with plant extracts after 24 hours.

Leaf Strength.

The results of tests of tensile strength using fifty 5-cm sections cut from each ryegrass cultivar are summarised in Figure 5. The annual ryegrasses, including the hybrid Manawa, were weakest, registering tensile strengths of less than 150 g/mg. The perennials and hybrid Ariki were stronger, with tensile strengths between 150 and 200 g/mg.

Table 9

Feeding Preference of Adult Argentine Stem Weevil When Offered a Choice of Two Regions of Leaf Tissue from Various Endophyte-free and Endophyte-infected Ryegrasses.

	% Weevils Showing a Feeding Preference #			
Leaf Region	Endophyte-free leaf preferred	Endophyte-infected preferred	No preference	No feeding
Ariki tips	30.0 ns	30.0	40.0	0
" "	32.5 ns	22.5	40.0	5
" "	30.0 ns	27.5	42.5	0
Nui tips	60.0 *	22.5	12.5	5
" "	67.5 *	20.0	12.5	0
" "	62.5 *	20.0	17.5	0
Nui bases	87.5 **	0	5.0	7.5
" "	95.0 **	0	2.5	2.5
" "	100.0 **	0	0	0

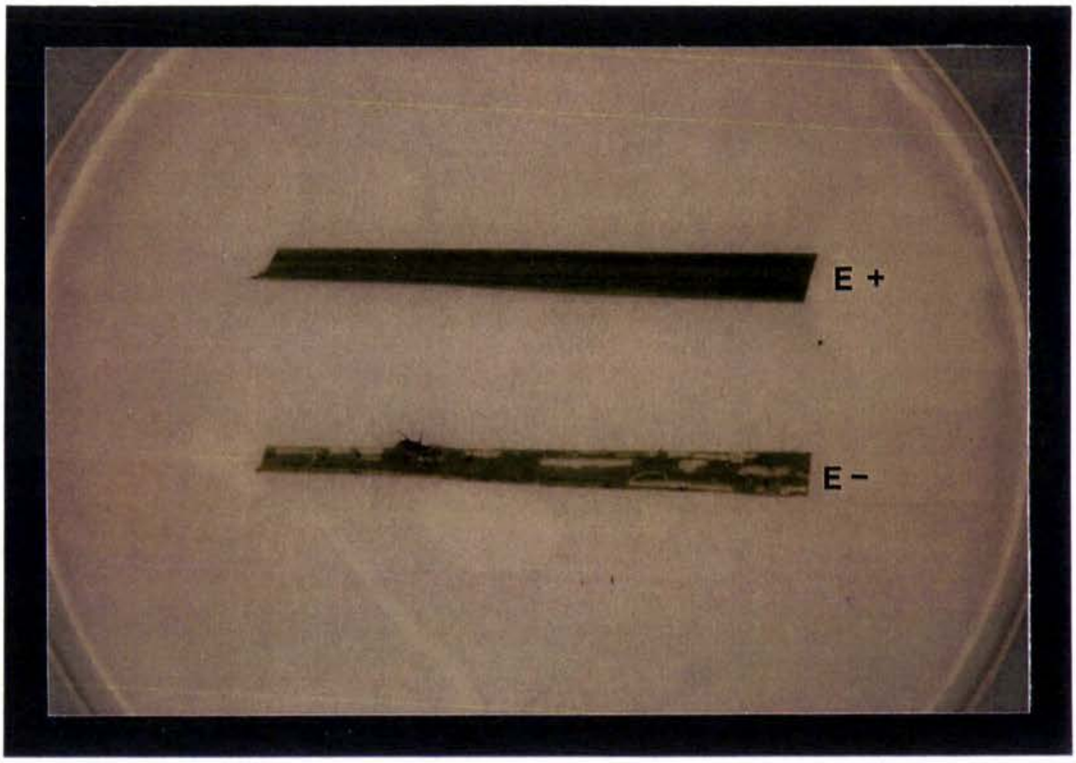
Forty adult weevils were used in each separate test.

ns = not significant

* = significantly different at the $P < 0.01$ level.

** = significantly different at the $P < 0.001$ level.

Plate 2



An example of a petri dish feeding bioassay.
The amount of feeding by one adult ASW after 12 hours on
endophyte-infected (E+) and endophyte-free (E-)
Nui ryegrass leaf base tissue.

Table 10

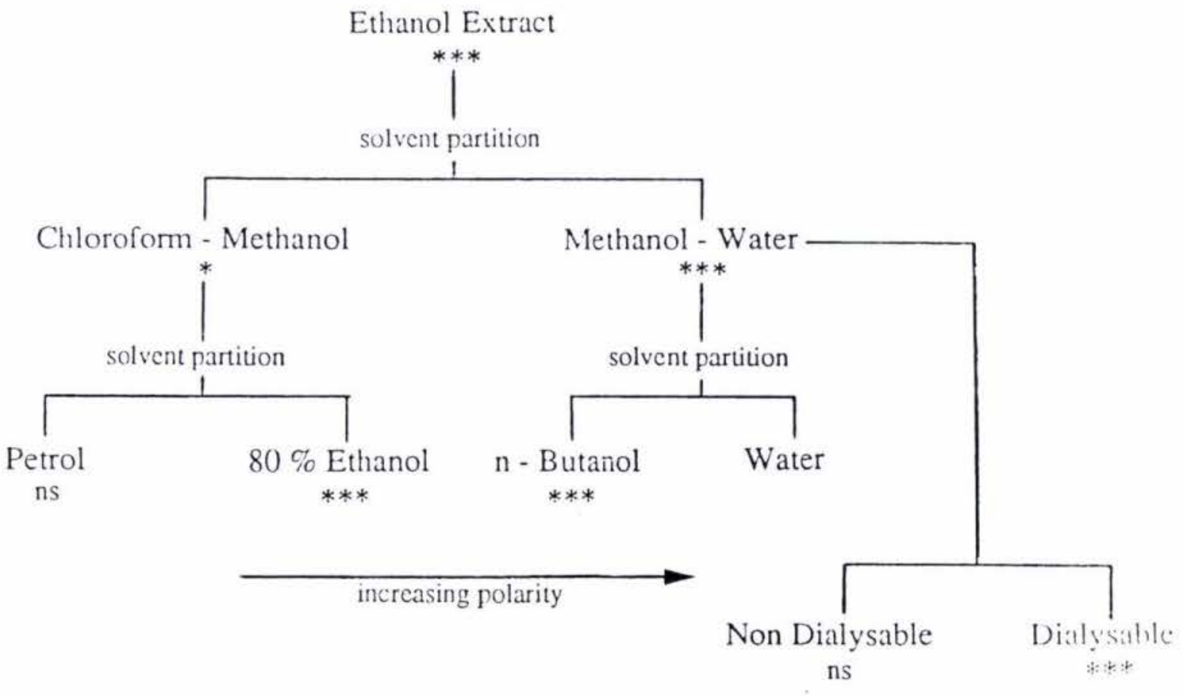
Feeding Preferences of Adult ASW When Offered a Choice of Agar Discs Containing Crude Extracts From Endophyte-free and Endophyte-infected Ryegrass.

Number of ASW / test	% Weevils Showing a Feeding Response			
	Endophyte-free disc preferred	Endophyte-infected disc preferred	No preference	No feeding
40	62 **	12	10	16
40	68 **	12	8	12
40	47 **	17	10	26

** = significantly different at the $P < 0.001$ level.

Figure 4

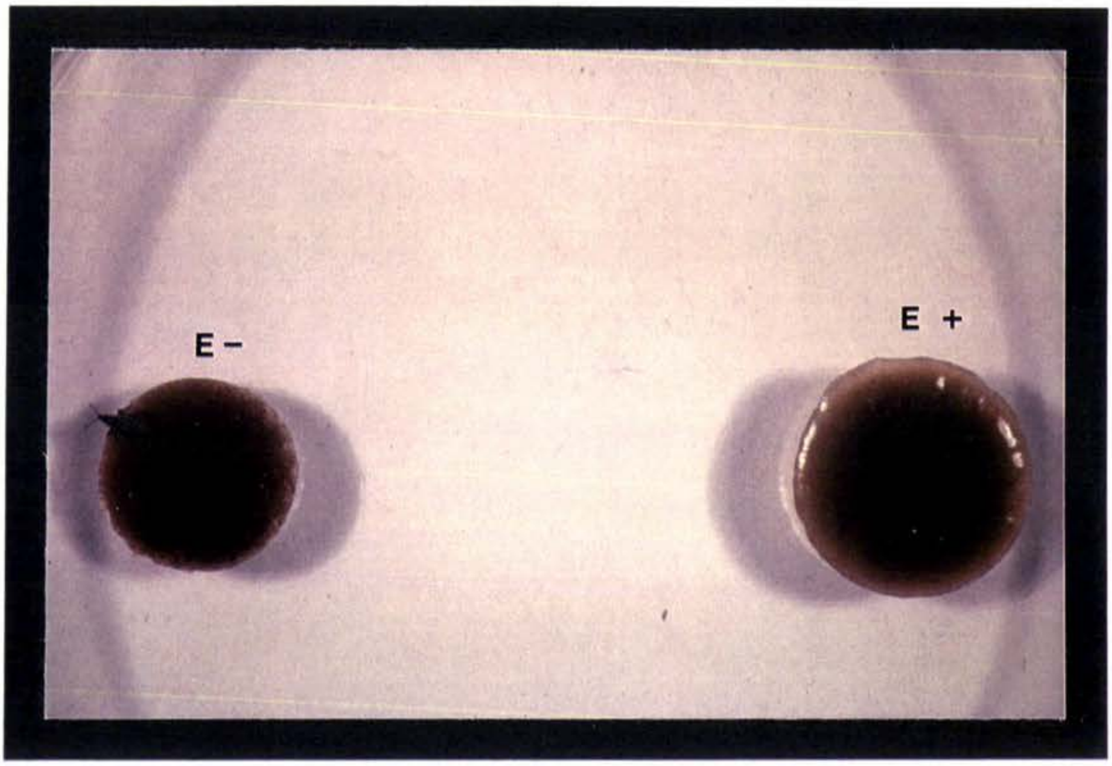
Scheme Showing Partitioning of Extracts from Endophyte-infected Ryegrass and Results of Bioassays of Feeding Preference to Partitioned Fractions.



Significant reductions in weevil feeding in choice tests are indicated as :-

ns = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Plate 3

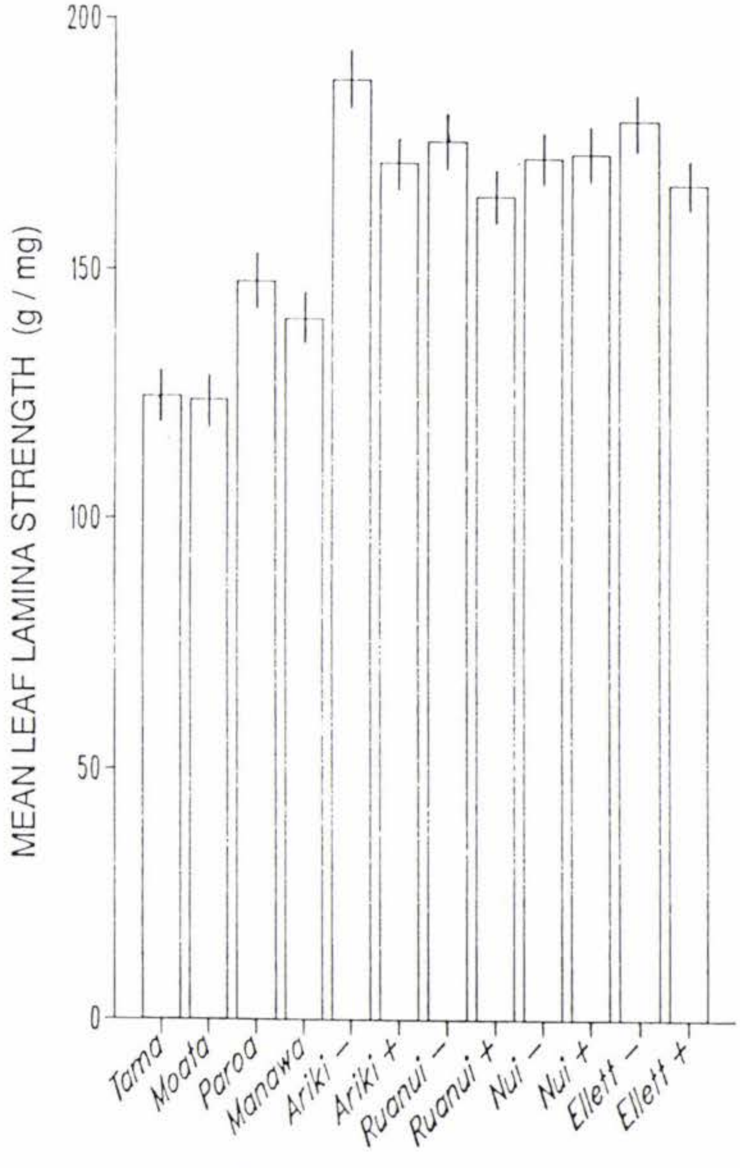


An example of a petri dish feeding bioassay.

The amount of feeding by one adult ASW after 24 hours on agar discs which contain crude extracts from endophyte-infected (E+) and endophyte-free (E-) Nui ryegrass leaf bases.

Figure 5

Mean Leaf Lamina Strengths of Various Ryegrass Cultivars.



Standard Errors are indicated by vertical lines on the columns.

Fibre Content.

The values obtained from analysis of fibre content for each cultivar are shown in Figure 6. No statistics are presented as there was insufficient tissue for repeat tests. However, the results suggest that Ellett cultivars contain the most fibre, followed by the hybrid Ariki's, then the other perennials, and lastly the annuals and hybrid Manawa with the least amount of fibre. The presence of endophyte does not appear to influence the percentage of fibre within the tissues, as values obtained from the perennial cultivars with and without endophyte are very similar.

Effects of Adult Size, Sex and Age on Feeding.

Large weevils ate more than small weevils. The mean number of feeding scars for large males was 5.9, and for large females 6.1, while for small males the mean was 5.5 and the small females 5.1. However, all pairwise comparisons were not significant (Mann-Whitney U-test).

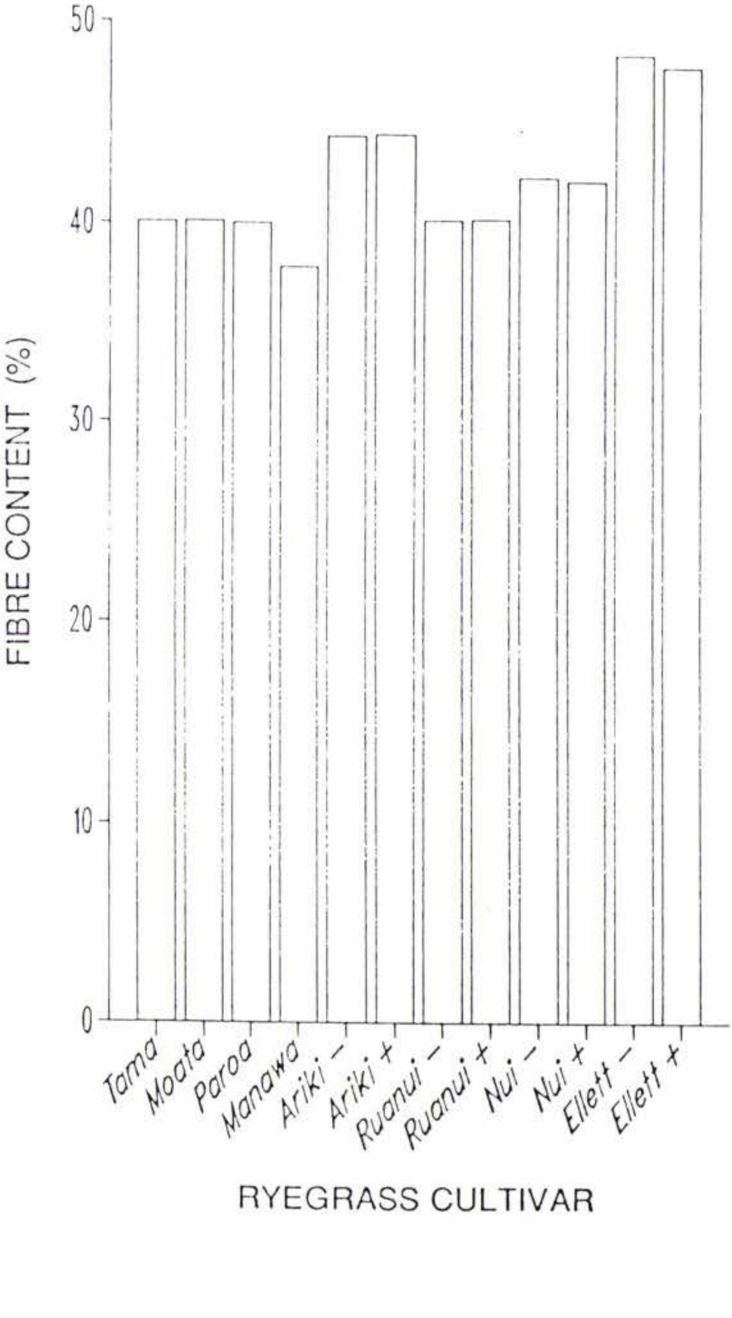
With respect to the age of the weevils, mean feeding scores were 5.25 and 4.15 for young and old weevils respectively. The difference, however, was not significant (Mann-Whitney U test).

Behavioural Observations.

In a no choice situation with an endophyte-free Nui perennial ryegrass leaf base, the weevil walked around the petri dish until encountering the leaf tissue. During this "exploratory" behaviour, the antennae were waved continuously, possibly to monitor odour with chemoreceptive sensilla on the antennae (Chapter 6). After contacting the leaf, the weevil walked over part of the tissue and took a "test" bite. Here both tarsal and mouthpart receptors (Chapter 6) came into direct contact with the leaf tissue. In all four tests on this susceptible ryegrass tissue, weevils continued to feed uninterrupted. They adopted a characteristic feeding posture; the antennal clubs touched the plant surface, the legs were anchored firmly about the leaf edge, and the head was lowered down between the forelegs to eat the plant tissue.

Figure 6

Total Fibre Content of Leaf Lamina Tissue of Various Ryegrass Cultivars.



Three of the four weevils restricted to endophyte-infected Nui perennial ryegrass leaf base tissue exhibited the same "exploratory" behaviour as above, took a test bite from the leaf tissue, then resumed the "exploratory" behaviour. Each time the tissue was encountered a "test" bite was taken. The fourth weevil immediately adopted the characteristic feeding posture and began feeding upon the leaf.

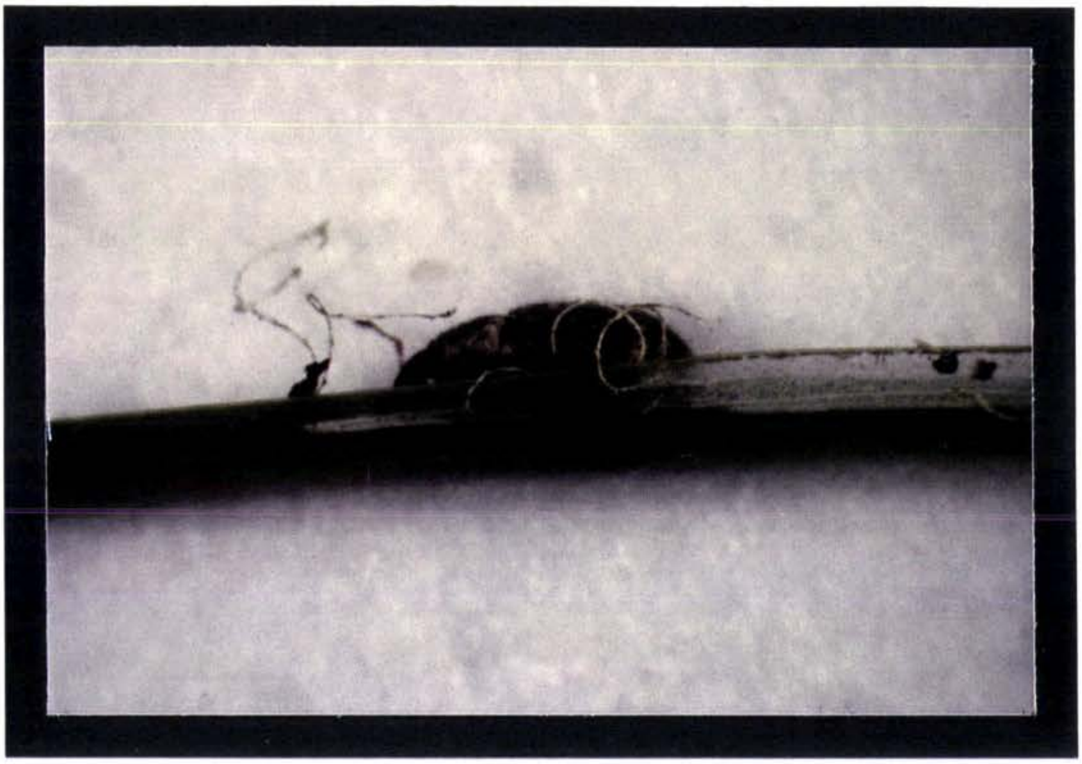
In the choice situation, a test bite was taken of the first plant tissue encountered, irrespective of endophyte content. If this tissue was endophyte-free, feeding continued, but if it was endophyte-infected, the weevil resumed "exploratory" behaviour until encountering the endophyte-free leaf where it fed continuously.

Weevil feeding damage shows up in the leaf tissue as characteristic whitish windows where tissue has been scraped off with the mandibles. There are also uneaten curled bundle sheaths (Plate 4).

DISCUSSION.

"All plants contacted elicit behaviour from phytophagous insects: some are rejected, some accepted. Although host-plants are in the latter category, they are not all equally preferred." (Hanson 1983). From the results presented in Figure 2 it is apparent that different ryegrass plants are not all equally preferred by ASW adults. The annual ryegrasses, Tama, Moata, and Paroa, plus the hybrid Manawa, were the most favoured cultivars for feeding. Perennials and other hybrids which were endophyte-free (Table 6) were next preferred and the endophyte positive cultivars least preferred. These results are similar to those obtained by Kelsey (1958), Pottinger (1961a and b), and Goldson (1979a), prior to the discovery of the relationship between endophyte incidence and ASW resistance (Mortimer *et al.* 1982, Prestidge *et al.* 1982). Taking into account the effect of endophyte, these results clearly show that where endophyte was present, ASW feeding was low (Fig. 2). The ELISA index values (Table 8), show that only sheath and basal tissue of endophyte-positive cultivars contain appreciable amounts of the fungus. From the tip region, only Ellett + and Nui + contain significant amounts of endophyte. All leaf regions of endophyte-minus and annual cultivars are endophyte free. Consistently, where endophyte levels were high, feeding damage was low (Fig. 2). This relationship is shown graphically in Figure 7.

Plate 4

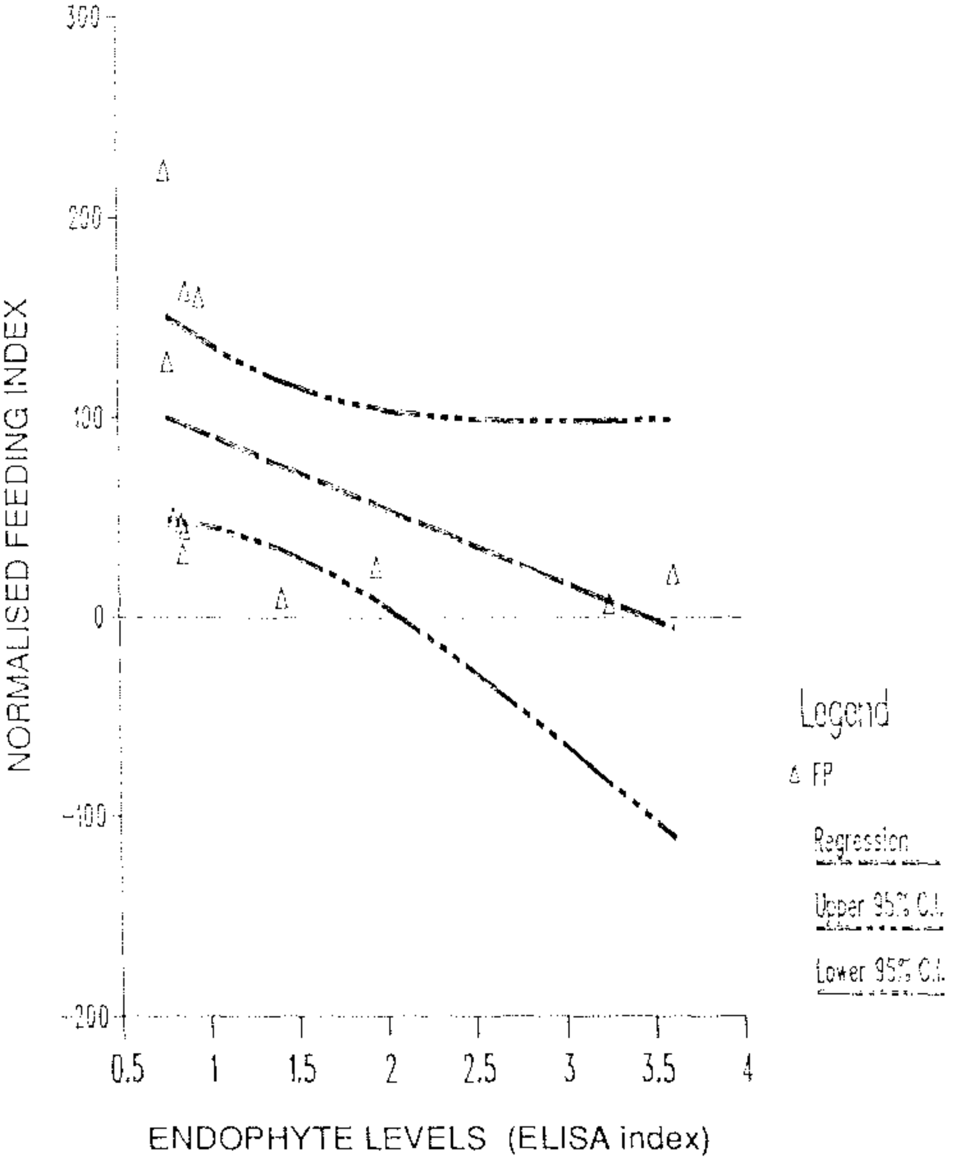


Characteristic ASW feeding damage on a susceptible ryegrass plant.

Note the curling of the bundle sheath to the left of the weevil and the window effect from tissue removed above the lower epidermis to the right.

Figure 7

The Relationship Between Argentine Stem Weevil Feeding Preference and Endophyte Incidence.



The Regression Equation is :-

$$Y = 129 - 37.1\ X \qquad n = 12, \qquad R^2 = 0.26, \qquad P < 0.05.$$

(Y = Feeding Preference, X = Endophyte Incidence).

The results obtained in the present study support the observations of Barker *et al.* (1983, 1984a and b), Prestidge *et al.* (1982, 1983, 1984a, 1985a, b, c and d), Gaynor and Hunt (1982) and Gaynor *et al.* (1983), that adult stem weevils prefer to feed upon ryegrasses free of *Acremonium lolii* endophyte. That ASW can discriminate between genetically identical ryegrass tissue which differs only in endophyte concentrations is clearly illustrated in the above sections; endophyte effects upon feeding and the feeding responses to various leaf regions (Table 9). Here, weevils were offered a choice of leaf tips from Ariki - and Ariki + ryegrasses and showed no feeding preference for either. This corresponds to the negligible amounts of endophyte within the leaf tip regions (both have insignificant amounts (Table 8)). More of a preference was exhibited for Nui - tips over Nui + tips, although feeding occurred on both, again paralleling endophyte incidence. Finally, where endophyte is concentrated in the basal region of Nui +, which has an ELISA index of 3.269 (Table 8), there is no feeding and the endophyte-free Nui leaf base was preferred (Table 9), which has an ELISA index of 0.808 (Table 8). This gradation in feeding preference parallels endophyte incidence, or some "cue" associated with endophyte infection. Leaf sheath material was not tested as feeding is normally restricted to the leaf lamina tissue. During oviposition, however, the female excavates an egg cavity with her mouthparts within the sheath region, and thus endophyte concentrations become important (see Chapter 5).

The reduced feeding of adult ASW on *A. lolii*-infected ryegrass tissue suggests the presence of a feeding deterrent. Such a deterrent has been demonstrated in this study (Fig. 4) and by Gaynor *et al.* (1983), Gaynor and Rowan (1985), Barker *et al.* (1983 and 1984a) and Rowan and Gaynor (1986), using a choice bioassay based on plant extracts incorporated into artificial diets. Furthermore, separation of the plant extract by solvent partitioning indicates that the active fraction is concentrated into the more polar phases of n-Butanol and water and not in the non-polar phases. Also, the deterrent is dialysable. This supports the conclusions of Gaynor *et al.* (1983), Gaynor and Rowan (1985) and Rowan and Gaynor (1986) that the deterrent is of low molecular weight and thus a highly polar hydrophilic compound. Complete partitioning to isolate the deterrent compound/s have been accomplished by Rowan and Gaynor (1986) and Gaynor and Rowan (1985). These authors have named the primary deterrent compound "peramine" ($C_{12}H_{17}N_5O$). Peramine deters adult weevil feeding at 1 ppm, a concentration comparable to that found in *A. lolii*-infected ryegrasses (Rowan and Gaynor 1986). Cultures of *A. lolii* endophyte have also been found to deter stem weevil feeding, and preliminary results show that they contain peramine

(Rowan pers. comm. 1986). This suggests that peramine is a fungal metabolite and not a phytoalexin. Prestidge *et al.* (1985d) have also reported a stem weevil feeding deterrent from cultures of *A. lolii.* The deterrent is also water soluble and therefore easily transported around the plant (Prestidge pers. comm. 1987). Weevils could detect this deterrent when 0.05% w/w mycelium was incorporated into an agar-based diet, but as yet no pure material has been isolated by these workers. Another adverse effect of endophyte upon ASW is that fewer eggs are found in the calyces of female weevils when they are confined for 96 hours on endophyte-infected ryegrasses, compared to those on endophyte-free plants (Barker *et al.* 1984b). Also fewer early instar larvae survive on endophyte-infected plants (Barker *et al.* 1984a).

The behavioural observations of weevils feeding in no choice situations with either endophyte-free or endophyte-infected Nui perennial ryegrass tissue confirmed the suppositions made in Chapter 3, that a touch/taste system operates (rather than an olfactory one) in the selection of a suitable host-plant by ASW. The stimuli do not appear to be volatile chemicals as they require contact by the insect before detection. However, olfaction cannot be totally ruled out (see Chapter 3).

The observation of one weevil feeding continuously when confined to endophyte-infected leaf tissue suggests that if a weevil is hungry, it will eat, even if the material is generally unsuitable. Both feeding and oviposition by adult ASW has been observed on endophyte-infected ryegrass in both choice and no-choice tests (Barker *et al.* 1983; Gaynor *et al.* 1983). Those weevils which are able to feed, reproduce and develop on endophyte-infected pastures are at a selective advantage in endophytic pastures, and thus may eventually give rise to endophyte-resistant pest populations (Dymock and Hunt 1987).

While not investigated in the present study, the age of a leaf is important in determining resistance to an insect herbivore. In an endophyte-infected ryegrass plant, the new leaves from an established tiller initially outgrow the fungus and remain free from its effects for up to ten days (Fletcher 1986). This effect is important, for example in fall armyworm (*Spodoptera frugiperda* (J.E. Smith)), larval preferences between fungal endophyte-infected and uninfected tall fescue, *Festuca arundinacea* Schreb., at three leaf ages were examined by Hardy *et al.* (1985). They found that neonate larvae did not discriminate between infected and uninfected new growth, but with older leaves there was a significant preference for uninfected over infected leaves. The plant factors related to leaf age which could potentially affect fall armyworm preference and development were determined by investigating the percent wet weight, water-soluble carbohydrate and crude protein content between infected and uninfected fescue leaves. Results indicated that water-soluble carbohydrate and crude protein content did not differ between infected and uninfected leaves at any leaf age, although crude protein was significantly higher, and water-soluble carbohydrate significantly lower, in young leaves. Both had no effect on feeding or development. However, old leaf blades had a significantly lower percent wet weight than younger leaf blades, irrespective of endophyte status, while older uninfected leaf blades contained more water than equivalent-aged infected leaf blades. Thus the larvae, when choosing to feed on endophyte-free leaves in a choice situation of either infected or uninfected old leaves, could respond to either a lack of deterrent factors associated with the endophyte, or to a preference for water, as influenced by pretest conditions.

The endophyte in tall fescue, *Acremonium coenophialum* Morgan-Jones and Gams (Morgan-Jones and Gams 1982) is thought to be identical or very closely related to *A. lolii* (Johnson *et al.* 1985). The implication of a fall armyworm preference for endophyte-free leaves which have a high water content over those infected with endophyte and a lower water content could be similar for ASW with respect to olfaction and the possible over-riding of these olfactory cues by moisture as discussed in Chapter 3.

A further *Acremonium* endophyte has recently been isolated from an annual (*L. multiflorum*) ryegrass cultivar (Christensen pers. comm. 1986). This endophyte is found only in the bottom 1 - 2 mm of the apical meristem region and in the nodal tissue of flowering stems and thus will rarely be encountered by feeding or ovipositing adult ASW. The effects of this endophyte on the plant or on feeding weevils have yet to be determined (Christensen pers. comm. 1987).

While endophyte presence/absence explains many of the earlier conflicting results, Gaynor and Hunt (1983) found that where there were low levels of endophyte, ryegrass cultivars with a *L. multiflorum* X *perenne* genotype such as Manawa were more susceptible to ASW than cultivars with a *L. perenne* genotype. These differences did not therefore appear to be due to endophyte. The present results from both feeding trials and endophyte incidence tests also support this conclusion, with all the endophyte-free cultivars producing various amounts of feeding, some significantly different from others. Gaynor and Hunt (1983) did not attempt to explain such differences other than to call them "genotypic effects." The leaf strength and fibre tests were undertaken to try and elucidate possible genotypic differences. Results of the leaf strength tests suggest a tendency for the perennial ryegrass cultivars to be tougher than the annual cultivars. Evans (1964) pointed out that fibre content is correlated to leaf strength, while Langer (1973) states that fibre imparts toughness to ryegrass leaves. The regression equation obtained from my results between fibre content and leaf lamina strength is:-

$$Y = 27.3 + 0.0935\,X \quad n = 12,\ R^2 = 0.37,\ P < 0.01.$$

(Y = Fibre Content, X = Leaf Lamina Strength).

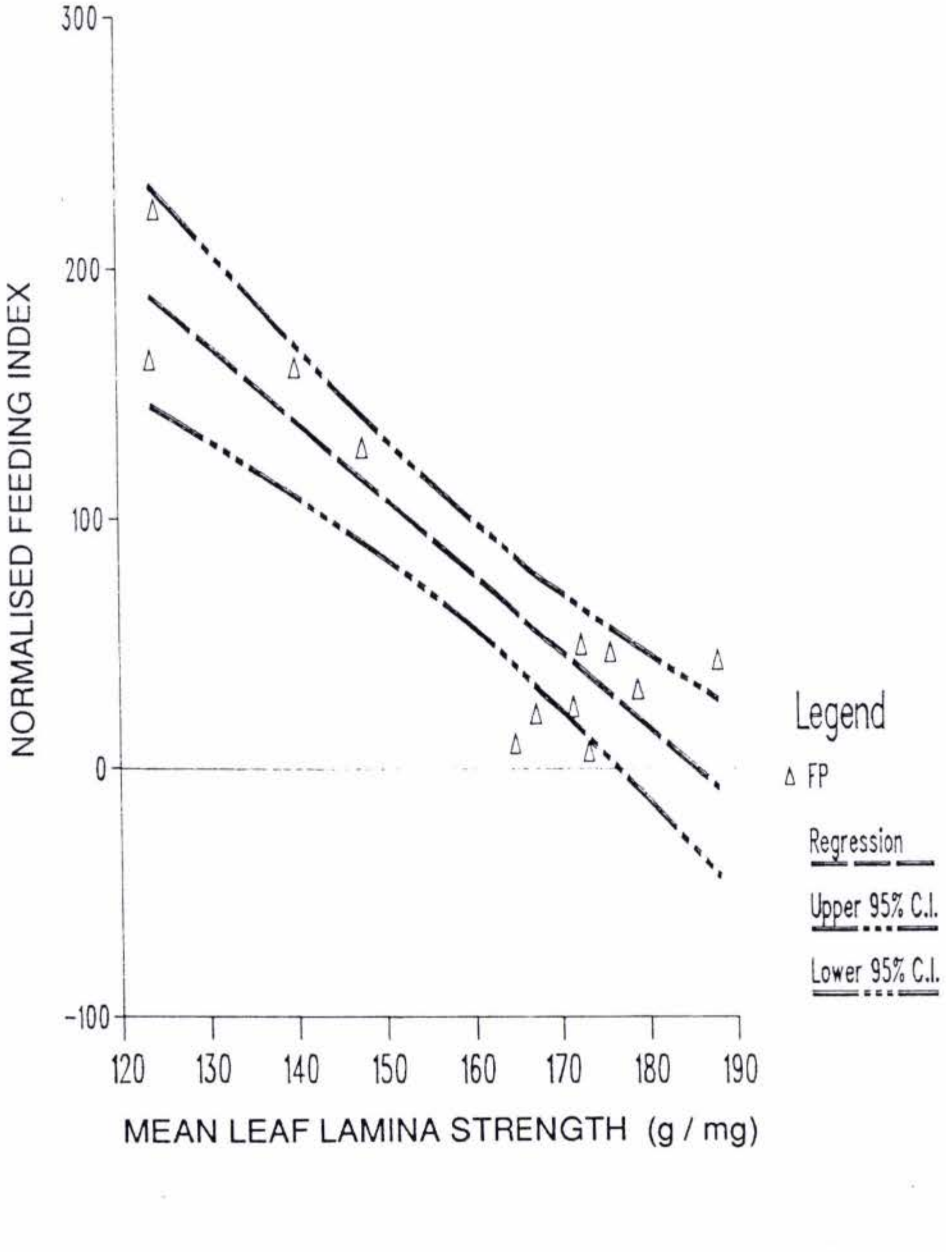
It is reasonable to suggest therefore that the observed inverse relationship between leaf strength and susceptibility may be related (in part) to the ease with which the weevil can penetrate the tissue in order to feed. The relationship between leaf lamina strength and ASW feeding preference is shown graphically in Figure 8.

Other genotypic characteristics may also influence the preferential feeding of ASW on the annuals over the perennials. Perennial ryegrass leaf blades are usually less than 14 cm long, and between 2 and 4 mm wide, while annual ryegrasses leaf blades are between 11 and 22 cm long and 3 and 8 mm wide (Langer and Hill 1982). Thus annuals tend to have less fibre per unit area than perennials, and therefore have a greater surface area available for ASW feeding without the toughness associated with fibre content. Additionally the wider blades of annuals may provide a better grip for feeding weevils, and possess more feeding stimulants.

While environmental and plant nutritional factors have been kept constant in the tests reported here, there is evidence that certain cultivars (e.g. Ruanui) grown under conditions of non-limiting supplies of nitrogen and water suffer more ASW damage (Gaynor and Hunt 1982). This could be due to nitrogen applications promoting rapid growth and thus reducing the concentration of endophyte mycelium (Keogh 1986; Stewart 1986). Gaynor and Hunt (1982, 1983) and Hunt and Gaynor (1982) have also stated that variability in susceptibility

Figure 8

The Relationship Between Argentine Stem Weevil Feeding Preference and Leaf Lamina Strength.



The Regression Equation is :-

$Y = 570 - 3.07 X$ $n = 12$, $R^2 = 0.81$, $P < 0.001$

(Y = Feeding Preference, X = Leaf Lamina Strength).

between perennial ryegrass cultivars as obtained by Kain *et al.* 1977, 1982; Goldson 1979a and b; and Lancashire 1977, could be influenced by environmental factors. For instance, ASW has long been recognised as a major pest of pastures in drought-prone areas. Drought causes reduced leaf expansion and tillering, an adaptive advantage for survival (Korte and Chu 1983; D.J. Barker *et al.* 1985). Therefore ASW infestations cause both established and replacement tillers to be destroyed (Percival and Duder 1983; Prestidge *et al.* 1985c). There is also differences between farms in levels of ASW populations and in damage. This suggests an interaction between soil type and grazing management in the expression of ASW damage (Barker *et al.* 1986). Furthermore, it is possible that some endophyte-infected cultivars produce more of the deterrent compound/s than others, or as Latch states (Latch pers. comm. 1987), different strains of *A. lolii* exist which may differ in their ability to produce the deterrent compounds. Prestidge *et al.* (1985b) have shown that either of these possibilities could be true, as equal concentrations of endophyte mycelium in three different cultivars, produced three different degrees of feeding on leaves. Similarly, cultures of some isolates appear to be more deterrent than others (Prestidge *et al.* 1985b). In the tests performed above, however, I assumed that the same amount of deterrent compound is produced per quantity of hyphal tissue in each cultivar, irrespective of possible strains.

In all tests, variables of the insect must be considered, in particular, age, sex, size, peak activity period, physiological and reproductive state plus behaviour (Smith 1978). With respect to sex, for example, in tests with crude white clover bud extract, significantly more female clover head weevils were attracted than males (Smith *et al.* 1976). Conversely, with the cotton boll weevil, more males were attracted to cotton oil baited traps than females (McKibben *et al.* 1977). The simple tests reported here, taking into account size and sex, suggest that feeding was proportional to body size. Smaller weevils ate less than larger weevils, but the difference was not significant. Likewise, differences between the sexes was not significant. It has been shown that male and female ASW do not differ in their degree of preference for endophyte-free plant material (Barker *et al.* 1984c). Therefore, provided samples are taken randomly from field populations, experimental bias should not occur with respect to size and sex.

Tests on the effect of age on weevil feeding, while not conclusive, showed a trend similar to that obtained by other authors in tests with alfalfa weevils (Koehler and Gyrisco 1963; Barnes and Ratcliffe 1967), in that younger weevils consumed more leaf material than older weevils. Koehler and Pimmental (1973) found that 5-day-old alfalfa weevils consumed six times more foliage than 20-day-old adults. Age in the tests I performed was not determined accurately, nevertheless, it may be appropriate in future to use young adults to ensure maximum responses in feeding tests. It may also be desirable to ascertain whether the weevils are from the overwintered diapausing second generation adults from the previous season, or whether they are the current season's first generation adults. It is possible that intensity of feeding could differ significantly between these groups. Overwintered adults live 8 to 9 months and lay their eggs in spring, which then develop into the "first summer generation" of larvae. Adults from this first generation live for only 4 to 6 months, and their progeny form the "second summer generation", which again overwinter and repeat the cycle (Kelsey 1958 and Pottinger 1961a, 1977). There is evidence that adult feeding declines rapidly after egg laying because of reduced metabolic demand (Goldson and Penman 1979), a further argument for using only young adults in tests. Observations of adult weevil feeding behaviour tend to contradict the results of leaf tensile strength and fibre content experiments. These tests conclusively showed that ASW preferred cultivars which were less tough and have a lower fibre content. However, feeding weevils avoid the large bundle sheaths present in the leaf lamina by selectively feeding between them, and it is the bundle sheath which harbours the majority of fibre (Esau 1960). These bundle sheaths become characteristically curled (Plate 4), a diagnostic feature of adult ASW feeding (Kelsey 1958, Pottinger 1961a, b). Additionally to enable inter-cultivar comparisons to be made, all cultivars were grown and tested at the same time of the year. However, annual ryegrasses are noted for their rapid establishment, vigorous growth and high yielding qualities in a short period of time. This extreme vigour is a decided practical advantage, hence their main use is to provide winter and early spring feed following autumn sowing (Vartha 1971). Provided sowing times coincide with ASW reproductive diapause, no significant damage to these cultivars results, even though they are highly susceptible until the following spring larval generation (Goldson and Penman 1980). On the other hand, the perennials are long lived and capable of producing many tillers. Their annual growth starts late in spring, with very high late summer and autumn production. Their greater tillering is a practical aspect of weevil resistance, called tolerance, which is the ability to tiller and grow under conditions of high weevil activity.

In summary:-

Feeding preferences of ASW are related to both the amount of endophyte within the leaf tissue (endophyte incidence) and leaf lamina strength. These relationships are shown graphically in Figures 7 and 8. Multiple linear regression analysis gives the best linear fit for this relationship as:

$$Y = -21.1\,X - 2.81\,Z + 557$$

where both X and Z are significant at $P < 0.01$, $n = 12$
(Y = feeding preference, X = endophyte incidence, and Z = leaf strength).

89% of the variability in Y is accounted for by X and Z.

CHAPTER 5

OVIPOSITION

INTRODUCTION.

Damage caused by ASW is mainly attributable to the larval stage, yet as previously mentioned in Chapter 1, it is the discriminatory behaviour of the ovipositing female in locating and selecting suitable host-plants which ultimately determines this damage. The strong selection pressure invoked by losses through indiscriminate egg laying mean that adult females of most insect species are usually fastidious in their choice of oviposition site. An investigation of oviposition responses and the role of plant characters determining these responses should therefore provide detailed information with respect to possible future managerial strategies for ASW.

In the vegetative condition (when most damage is done), ryegrass plants consist of a collection of shoots or tillers. A tiller comprises a leaf blade or lamina and a leaf sheath which arises from nodes at the base of the tiller. Oviposition involves the female firstly chewing a small hole in the epidermis of the leaf sheath close to the ground, and then depositing between 1 and 6 eggs into this hole (Blair and Morrison 1954, Pottinger 1961a). Many authors (Kelsey 1958; Pottinger 1961a; Timlin 1964; Prestidge *et al.* 1982) noted that short-rotation and Italian ryegrasses attracted more oviposition than perennial ryegrasses, and Goldson (1982) even suggested that susceptibility of a ryegrass cultivar to egg laying is proportional to how much of its genetic component is short rotation (*Lolium multiflorum*) rather than perennial (*L. perenne*). A selection of all available ryegrass cultivars were thus examined for ASW oviposition preference (or non-preference). After these oviposition preferences had been determined, direct behavioural observations were carried out in the laboratory using the most susceptible and the most resistant cultivars. These observations not only enabled a sequence of ovipositional events to be defined for ASW for the first time, but also established the underlying responses that determine selection of an ovipositional site on a resistant or susceptible ryegrass cultivar.

One of the plant characters which has been shown to influence behavioural responses during oviposition is the fungal endophyte *Acremonium lolii* Latch, Christensen and Samuels (Latch *et al.* (1984), (Mortimer *et al.* 1982; Prestidge *et al.* 1982; Gaynor and Hunt 1983; Barker *et al.* 1983, 1984c; and Prestidge *et al.* 1985c). The amount of endophyte infection within the ryegrass cultivars selected for testing in this study was therefore determined.

Another possible plant characteristic affecting ASW oviposition is tiller diameter. Pottinger (1961a) briefly mentions that egg laying occurs only in stems which are greater than 1 mm in diameter. In the USA a positive linear correlation was shown to exist between stem diameter and number of eggs laid for the alfalfa weevil - *Hypera postica* (Gyllenhal), the smaller diameter stems receiving the least (Webster 1912; Sweetman 1929; Campbell and Dudley 1965; and Norwood *et al.* 1967a and b). The most extensive results are from Van Denburgh *et al.* (1966) who evaluated stem diameter, stem age, and stem hardness of alfalfa clones. in relation to the oviposition and feeding of the alfalfa weevil. These parameters were therefore investigated in the present study with ryegrass cultivars. The results of tiller diameter and tiller hardness tests were backed up with observations using various diameter glass tubing to simulate different diameters of grass tillers. An analysis of fibre content of each ryegrass cultivar was also made because fibre content of grasses is correlated with tiller hardness or strength (Bailey 1964, and Langer 1973).

MATERIALS and METHODS.

Oviposition Preference Trials.

The ryegrass cultivars used are listed in Table 6. Each cultivar was grown from seed by sowing in seed trays in mid August 1985 following the methods outlined in Chapter 4. The plants were raised in a glasshouse until late October with natural light and temperature, and watered twice daily by capillary bed. Individual plants were then replanted into 12 cm^2 pots, and regularly cut with scissors to maintain good vegetative growth. In early January 1986, ten plants of each cultivar were trimmed to ten tillers, each pot was covered with a muslin netting, and 30 adult weevils introduced (10 males and 20 females). The incubation period of ASW is approximately 14 days (Goldson 1982), therefore these plants were left in the glasshouse for 12 days to allow the maximum numbers of eggs to be laid before any hatched. All tillers were then cut off about 5 cm below the soil surface and visually inspected for eggs.

Endophyte Detection.

As the leaf sheath is the preferred oviposition site, leaf sheath tissue from twenty tillers from various plants of each ryegrass cultivar were bulked together and tested using the enzyme-linked immunosorbent assay (ELISA) as outlined in Chapter 4.

Tiller Characteristics.

Age of Tillers.

Plants of the most susceptible ryegrass cultivar, the annual Tama (from results of the oviposition preference trial above), were cut back to the crown at weekly intervals to provide tillers with two to eight weeks of regrowth at the time of testing. Also, each plant was trimmed to five tillers. These plants were transferred to a weevil-infested Nui ryegrass paddock in mid January 1986, and left for five days before the number of eggs laid on them were counted.

Tiller Hardness.

An Instrom (model 1140) was set up as a penetrometer using a 1 to 20 N compression load cell. Ryegrass tiller tissue of equal age from each cultivar was tested at the sheath region, and the pressures required to penetrate the epidermal layer recorded.

Fibre Content.

Ten grams of fresh ryegrass sheath material from each cultivar was freeze-dried for 24 hours. This dry material was then finely ground in a mortar and pestle. One gram of each sample was then analysed for the total fibre content by the neutral-detergent fibre determination method, as described by Robertson and Van Soest (1963).

Tiller Diameter.

Diameters of 100 tillers of each ryegrass cultivar were measured with calipers (± 0.05mm).

Glass Rod Preference Trial.

For each test, 30 unsexed adult weevils food-starved for 24 hours were introduced into a clear plastic observation chamber (24 X 24 X 10 cm). The floor consisted of sand to a depth of 5 cm supporting upright glass rods of 1, 2, 3, 4, and 5 mm diameter. There were five rods of each diameter arranged in a Latin square design. A plug of nutrient agar (4% cellulose powder, 4% Davis agar and 5% sucrose and 90 ml of distilled water (Gaynor *et al.* 1983)) was impaled on top of each rod to retain weevils on them for as long as possible and thus facilitate counting. The number of weevils on each glass rod was recorded at 10 minute intervals for one hour. Tests were run indoors at night and observed with a "Varo Noctron V" night-scope and the available light. Each test was repeated five times.

Oviposition Behaviour.

As ASW oviposits only at night all visual observations were carried out in an enclosed arena within the laboratory using a "Varo Noctron V" night scope attached to a JVC colour video camera. Recordings were made over a 12 hour period with a National/Panasonic Time Lapse video recorder NV-8050 coupled to the camera by a JVC camera adaptor. The resultant video tape was viewed later on a CCTV monitor (model PM-900). From the results of the oviposition preference trials, one tiller from the most susceptible ryegrass (Tama), and one tiller from the most resistant (Ellett +), were used to observe the weevils' behaviour on consecutive nights. The plants were in 12 cm^2 pots and all but one tiller was removed. The whole pot was placed in the observation arena. The arena consisted of a cylinder of clear plastic, 15 cm diameter, closed at both ends with a petri dish which had 0.5 mm diameter holes drilled through for ventilation purposes. Initially one adult male and one adult female were introduced into the observation arena and left overnight, with the behaviour of the female only observed. However, because observations were infrequent within the field of view, especially oviposition behaviour, the numbers were increased to five of each sex, with five fresh ryegrass tillers. Furthermore, because of the small size of ASW and the necessity for precise behavioural examinations, the camera (and nightscope) was connected to an "Olympus" dissecting microscope. This restricted the field of view to a 5 cm diameter area, so the camera was adjusted to focus on the base of the ryegrass tiller, the first point of encounter by a weevil. The weevils were starved for 24 hours prior to experimentation, but had access to water. In all tests females were individually identified by painting an opaque dot at specific positions on their elytra. Similar marking of the alfalfa weevil had no effect upon the weevil's behaviour (Taft and Agee 1962). The addition of males to the test arena ensured that females were mated, but male behaviour was not recorded.

RESULTS.

Oviposition Preference Trials.

ASW oviposition preferences, measured as the number of eggs laid in 18-week old ryegrasses after 12 days are given in Figure 9. The annual ryegrasses Tama, Moata and Paroa, as well as the hybrid Manawa were the most preferred cultivars. Next were those cultivars designated as endophyte-free in Table 6, with the least preferred cultivars those designated as endophyte-positive. Plate 5 shows eggs deposited into the sheath region of Tama ryegrass tillers.

Endophyte Incidence.

The relative amount of endophyte present in each cultivar, expressed as ELISA indices, varied from 0.863 to 7.331 (Fig. 10). All annual and endophyte-minus cultivars had ELISA indices below 1.1. These can be considered endophyte-free because ELISA indices below this value indicate a 99% probability of there being no endophyte present (Musgrave 1984).

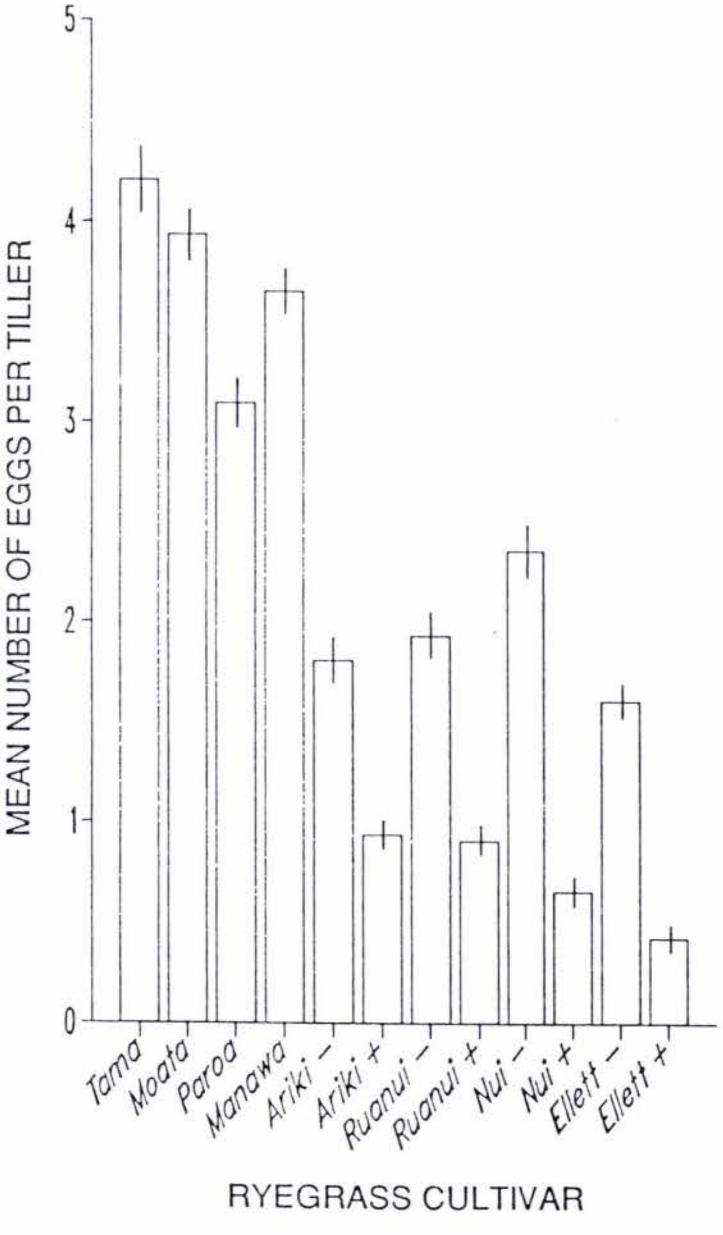
Tiller Characteristics.

Age of Tillers.

There were no significant differences between the numbers of eggs laid in relation to the age of ryegrass tillers (analysis of variance $F_{6,28} = 0.03$, NS). (Table 11).

Figure 9

Oviposition Preference of Argentine Stem Weevil for Various Ryegrass Cultivars.



Standard Errors are indicated by vertical lines on the columns.

Plate 5

ASW eggs (arrows) deposited into the leaf sheath region of Tama ryegrass tillers.

(Photo by V.A. Hunt)

Figure 10

Endophyte Levels from the Sheath Tissue
from Various Ryegrass Cultivars.

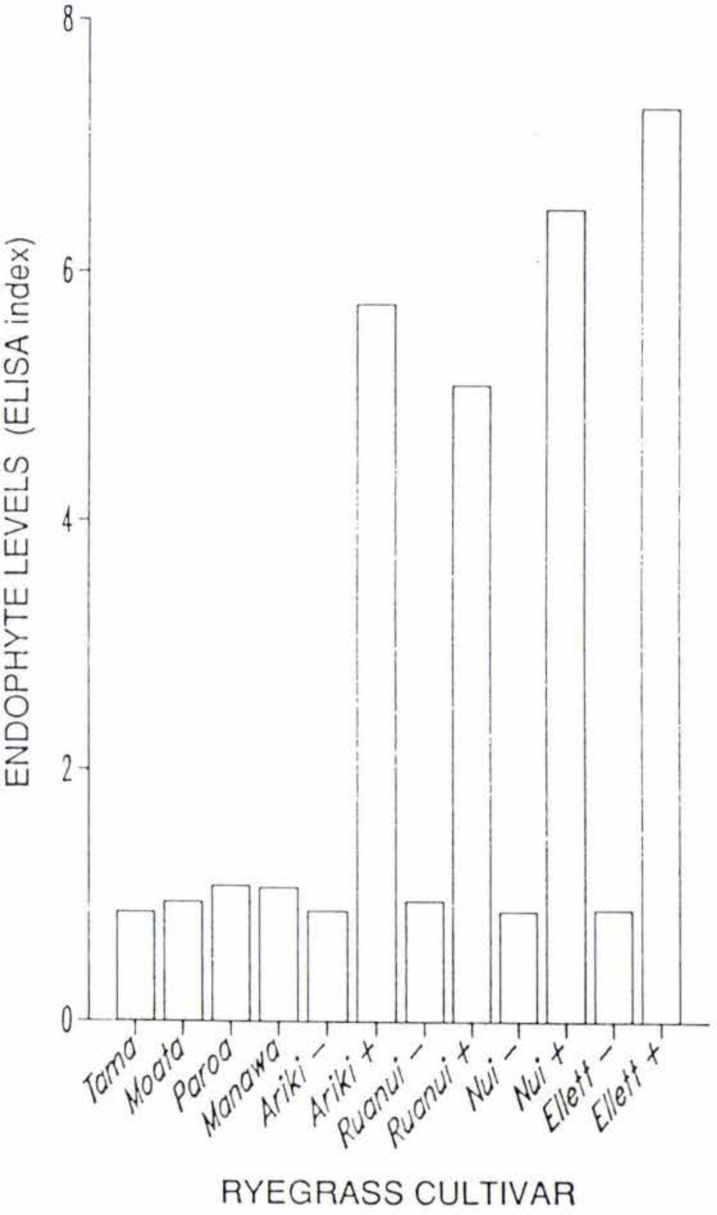


Table 11

Effect of Tiller Age on Oviposition.
(after field exposure for 5 days)

Initial Tiller Age (weeks)	Number of eggs laid per tiller					Total	Mean	± SE
2	6	3	1	7	4	21	4.2	1.1
3	3	6	5	5	2	20	4.2	0.7
4	2	7	6	1	4	20	4.0	1.1
5	1	9	3	3	3	19	3.8	1.4
6	6	5	5	1	3	20	4.0	0.9
7	6	1	7	4	4	22	4.4	1.0
8	1	6	6	3	5	21	4.2	1.0

Tiller Hardness.

All forces registered by the Instrom were below the lower limit of the smallest load cell available (i.e. less than 0.1g/mm^2) and therefore no effective readings of penetrability could be obtained. However, there was a trend for the annuals to be less resistant to the penetrometer probe than the perennials when the chart recording was examined visually, but values for both were so small that statistical analysis was inappropriate.

Fibre Content.

The amount of fibre from the sheath region of each ryegrass cultivar is given in Figure 11. Sufficient tissue to duplicate the fibre test was not available and hence no statistics have been performed on the results. However, Figure 11 suggests that the cultivars of Ellett contain the most fibre, followed by the hybrid Ariki's and other perennials. The annuals and hybrid Manawa contain the least amount of fibre. The presence of endophyte does not appear to influence the percentage of fibre within the tissue, as values obtained for the perennial cultivars with and without endophyte are similar.

Tiller Diameter.

Results are presented in Figure 12. The mean diameters of tillers ranged from 0.85 ± 0.02 mm to 2.055 ± 0.03 mm. Annuals (Tama, Moata, and Paroa) had the thickest tillers, followed by hybrids (Manawa, Ariki +/-). The perennials (Ruanui, Nui, and Ellett), with or without endophyte, were the thinnest.

Glass rod preference trials.

Over the 5 trials 69.5% of the weevils climbed the glass tubing and fed on the agar plugs. The results are expressed graphically in Figure 13 as the mean of all 5 trials and as a percentage of total weevils responding. Forty-five percent of the weevils responding preferred the 3 mm diameter glass rods. The larger 4 mm and 5 mm rods were next preferred with 30% and 12% respectively. The smaller two diameters of 1 mm and 2 mm were the least preferred with only 2% and 8% responding. All values differ significantly from each other ($P < 0.01$ (Tukey Test)).

Figure 11

Total Fibre Content of Sheath Tissue from Various Ryegrass Cultivars.

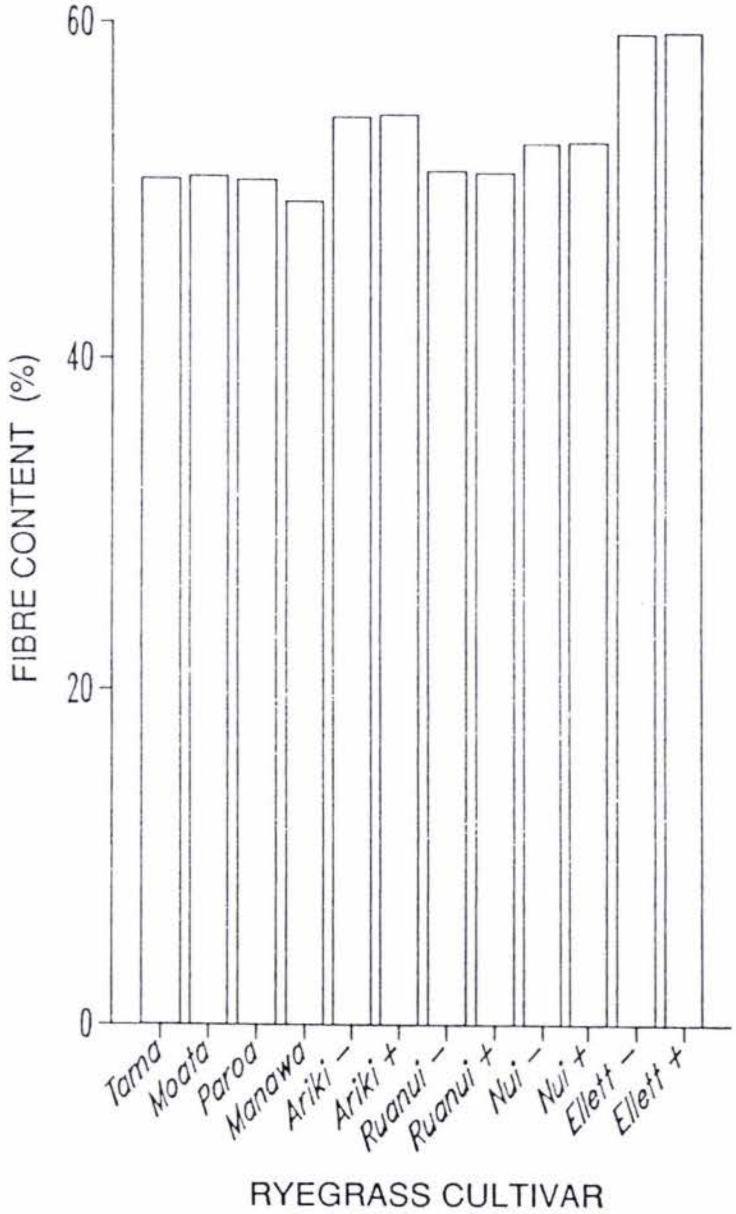
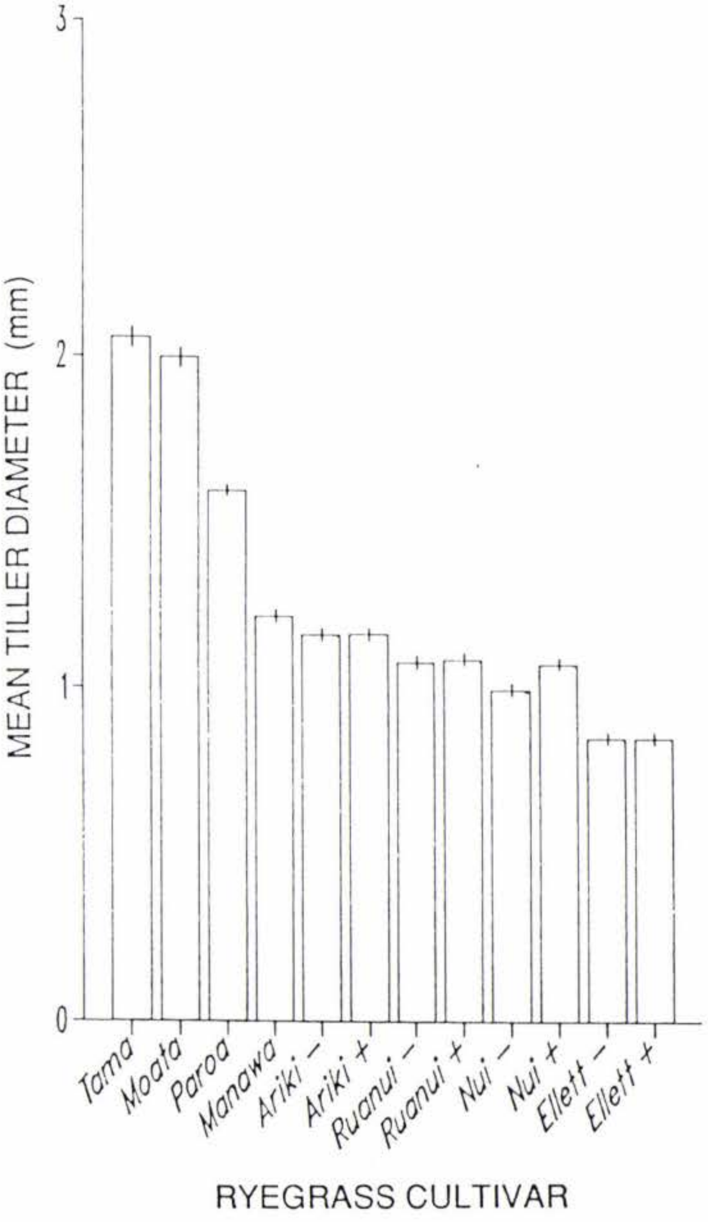


Figure 12

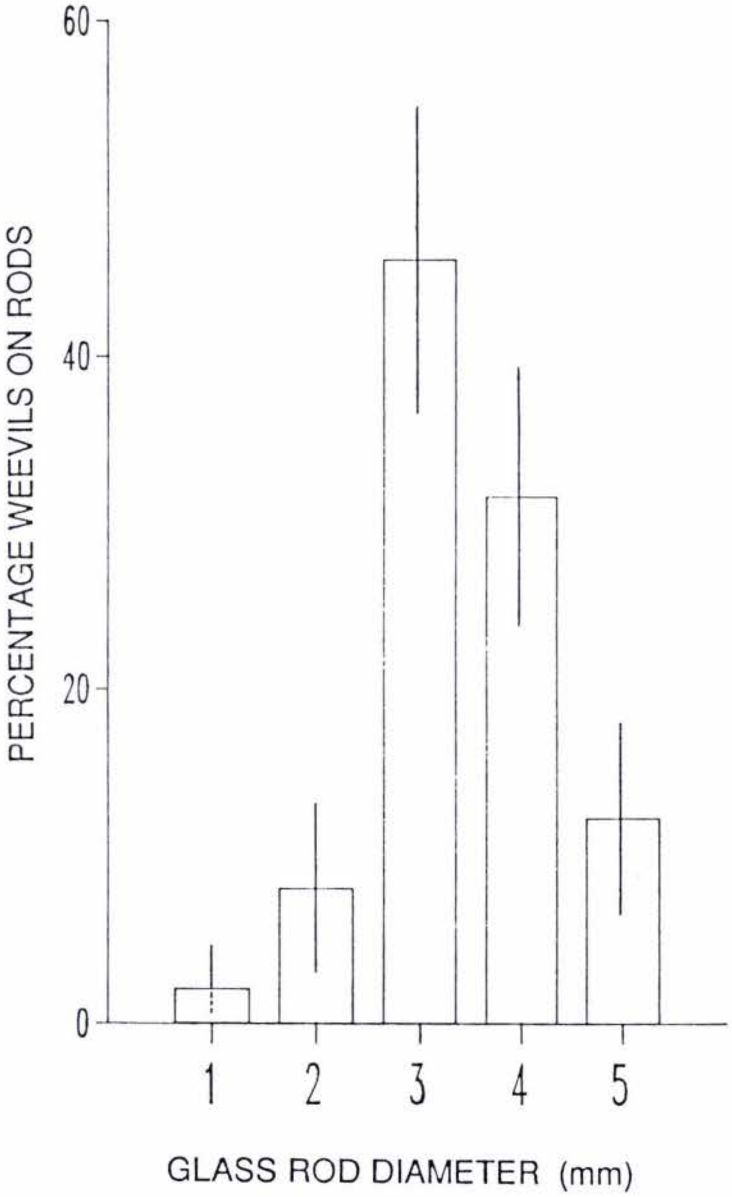
Mean Tiller Diameters of Various Ryegrass Cultivars.



Standard Errors are indicated by vertical lines on the columns.

Figure 13

Numbers of Argentine Stem Weevils Climbing Glass Rods of Various Diameters.



Standard Errors are indicated by vertical lines on the columns.

Oviposition Behaviour.

Results from the observation of female ASW behaviour transcribed from the video tapes are summarised in Table 12.

Where the behaviour of the adult female alone was followed, the times taken from initial entry into the arena until encountering the tiller were 4.13 minutes for the resistant cultivar, and 6.24 minutes for the susceptible cultivar. Amalgamating these with the tests where 5 adult females were introduced gives mean times of 4.30 minutes for the resistant cultivar, and 5.93 minutes for the susceptible cultivar. Times varied across both trials from 0.59 seconds to 10.49 minutes, thus there was no significant difference between the average times taken for a weevil to reach either the susceptible or resistant ryegrass cultivar (Mann-Whitney U Test).

The behavioural sequences observed after a female contacted either a susceptible or resistant ryegrass tiller varied little. Only the frequency of their occurrence (whether alone or with other females) varied. After contacting a tiller, females began to climb, often stopping within the first few centimetres. While stopped, they either tapped the tiller with their antennae or waved them in the air. On one occasion, a female may have sampled the tissue with her mouthparts because she adopted the characteristic feeding posture of adult weevils (Chapter 4), with legs firmly anchored about the tiller and head down between the forelegs. However, her body restricted a clear view. After this initial "exploratory" behaviour, weevils left the field of view by either climbing further up the tiller, or by walking down and out into the arena. The frequency of entering and leaving the field of view is also recorded in Table 12. When only one adult female was present at a time, the frequency of arriving at and leaving the resistant tiller was 163 times, compared to only 67 times for the susceptible tiller. Combining these with the results when 5 adult females were present, the mean number of times weevils left and/or arrived at the resistant tiller was 133 times, and for the susceptible cultivar, 54 times. Thus the weevils preferred to stay on the susceptible cultivar ($P < 0.005$. Mann-Whitney U Test).

Table 12

Female Weevil Behaviour at Different Densities of Adults on a Susceptible or Resistant Ryegrass Cultivar.

	1 Adult Female		5 Adult Females	
	Resistant Cultivar (Ellett)	Susceptible Cultivar (Tama)	Resistant Cultivar (Ellett)	Susceptible Cultivar (Tama)
Time taken from initial entrance into arena to encounter the tiller. (minutes)	4.1	36.24	4.3	5.93 # NS
Frequency of entering and leaving the field of view. (per 8 hour period)	163	67	133 #	54 # **
Number of eggs laid.	0	3	4	10
Number of punctures.	0	2	4 !	4
Number sealed with frass.	0	0	2	3

\# averages over the 5 trials.

! punctures in leaf lamina NOT sheath material.

NS = Not Significant.

** = $P < 0.005$. (Mann-Whitney U Test).

The actual process of oviposition was observed just once when 5 adult females were confined to the susceptible ryegrass cultivar. The components of this behaviour were as follows:

1) Tiller exploration - Before selecting an oviposition site the female weevil travelled up and down the tiller, entering and leaving the field of view 5 times. She occasionally stopped to bite the tissue and tap it with her antennae. Finally, she selected an oviposition site 12 minutes after contacting the tiller.

2) Egg cavity formation - After selecting a suitable site on the tiller, the weevil gnawed a hole into the epidermis. The legs were anchored firmly about the tiller. Pulsation, distension, and extrusion of the tip of the abdomen beyond the elytra occurred, and eventually extrusion of the ovipositor. This whole process took 3.36 minutes.

3) Turn - After egg cavity formation the female turned around.

4) Egg deposition - The tip of the abdomen/ovipositor was placed near the puncture. It was then moved in small arcs to locate the hole, then the ovipositor appeared to be extruded further and two eggs were deposited onto the inner epidermal tissue of the tiller.

5) Ovipositor retraction - After depositing the eggs, the female withdrew her ovipositor, which retracted into the body cavity.

6) Tiller abandonment - The weevil left the tiller.

Activities 4 to 6 took 1.06 minutes.

After each trial each tiller was removed from the observation arena and inspected visually for feeding and oviposition scars. When only one female was present, the resistant ryegrass tiller Ellett + had substantially less feeding injury than the susceptible Tama ryegrass tiller. No eggs were laid within the tissue of Ellett +, while Tama had three eggs laid within two oviposition punctures. When 10 adult weevils were enclosed, (5 of each sex), Tama had four oviposition punctures present in the sheath region and a total of 10 eggs. Three of these punctures were sealed with frass, the other was left unsealed. No eggs were present within the sheath material of the resistant cultivar Ellett +, but four individually deposited eggs were present under the leaf lamina epidermis. Two of these oviposition punctures were sealed with frass, the other two were empty. All these eggs were positioned within the last 4 cm of the leaf tip. Also, as before, the amount of feeding was substantially less upon Ellett + than Tama, with the feeding scars being confined to the tips of the leaf lamina in Ellett +, while the entire leaves of Tama were shredded.

DISCUSSION.

The results of the oviposition preference tests (Fig. 9) are very similar to those obtained by Goldson (1982) and Gaynor and Hunt (1983) in that they demonstrate a general preference for the short-rotation ryegrasses, Tama, Moata and Paroa. The hybrid cultivar Manawa was also highly preferred, probably because Manawa is a short-lived perennial with agronomic and botanical characteristics of it's annual parent (Langer and Hill 1982). The perennial cultivars, Nui, Ruanui and Ellett were least preferred. The Ariki hybrids were intermediate, which again probably reflects their genetic composition. Ariki hybrids are produced by Manawa being crossed with a perennial, and are thus closer in appearance and agronomic characteristics to the perennial rather than the annual parent (Langer and Hill 1982), (see Table 6 for genetic complement of cultivars). These results bear out Goldson's (1982) suggestion that susceptibility of a ryegrass cultivar to egg laying is proportional to how much of its genetic component is short-rotation (*L. multiflorum*) rather than perennial (*L. perenne*). Gaynor and Hunt (1983) also considered genotypic effects important but the actual factors determining oviposition preferences of annuals over perennials were not determined.

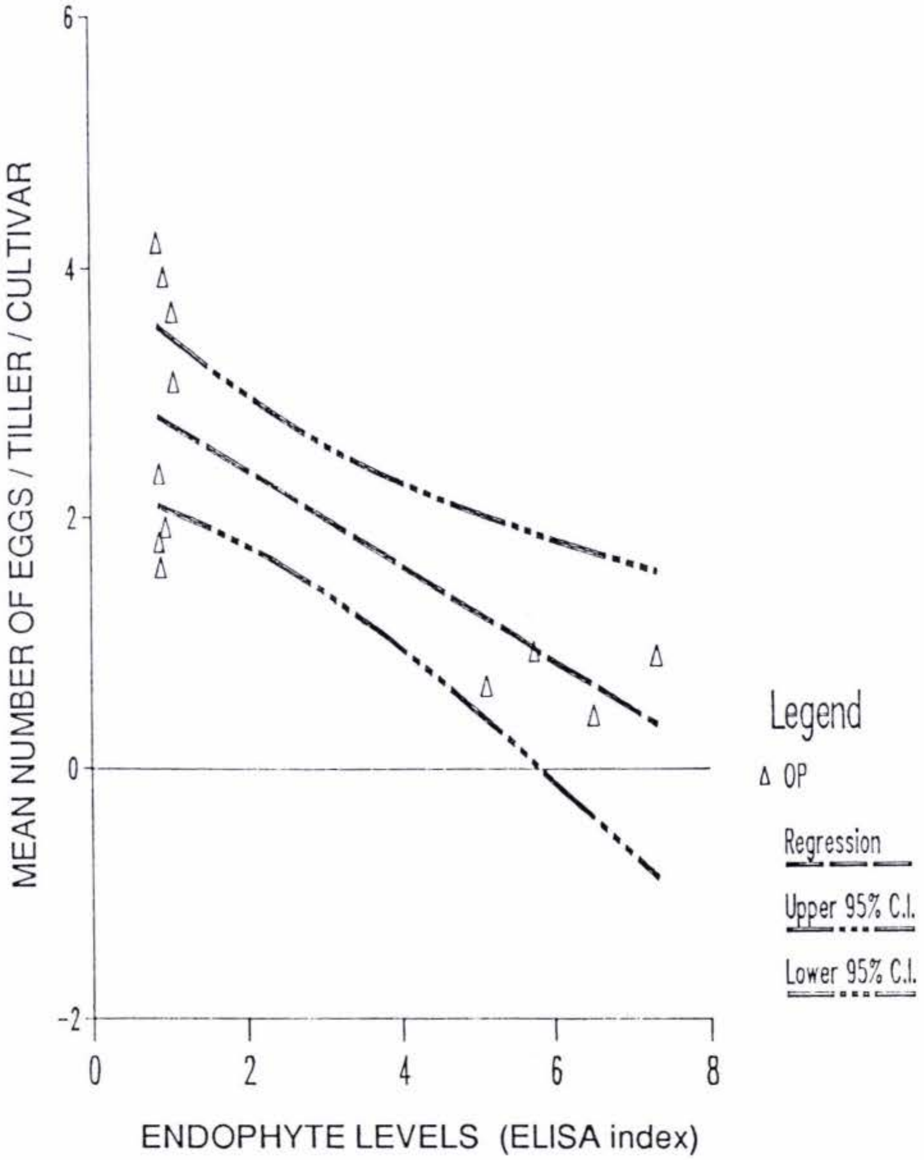
If the cultivars are divided into those containing endophyte (E+) and those free of endophyte (E-), it is clear that all endophyte-free cultivars are preferred for oviposition (Fig. 9). The results show that cultivars designated as containing endophyte in Table 6, (i.e. the hybrid Ariki + and perennials Ruanui +, Nui +, and Ellett +), all have significant proportions of endophyte hyphae within their tissue, while the annuals and other cultivars designated as endophyte-minus, contain none when tested by ELISA. Thus, in general, the greater the concentration of endophyte within the plant tissue, the fewer eggs are laid in it. Numerous authors have clearly demonstrated that ASW are extremely sensitive to endophyte presence. When count's of *A. lolii* hyphae per mm. breadth of leaf sheath exceed 10, feeding is restricted to "test bites" (di Menna and Waller 1986). This is in direct contrast to plants with no hyphal tissue present. Here more than 60% of the leaf is eaten (Prestidge *et al.* 1985b). The weevil can also detect mycelial concentrations as low as 0.05% w/w in artificial diet (Prestidge *et al.* 1985d). Reproductive activity of ASW is thus concentrated on the grasses which lack the fungus (Gaynor and Hunt 1983; Barker *et al.* 1983, 1984b, and c; Prestidge *et al.* 1985c, and this thesis). Female weevils confined for 96 hours on endophyte-infected plants contain fewer eggs in their calyces than weevils' kept on plants without endophyte (Barker *et* al. 1984c), although it has not been established whether this is due to biochemical interference with oogenesis or restricted nutrition.

It is also interesting to note that 76% of the total endophyte mycelium in vegetative tillers is located between 0 and 5 cm above ground and 44% is within the sheath region (Musgrave and Fletcher 1984). Thus the highest concentration occurs where the adult female weevil excavates an egg cavity by chewing through the epidermis. This would bring the mouthpart receptors into direct contact with the endophyte. However, it is uncertain whether the endophyte hyphal tissue itself or chemical compounds produced by either the fungus or the plant are responsible for the deterrent effects on oviposition. Certainly, the deterrent is not volatile, and can only be detected by contact and probing the plant surface. If one extrapolates from feeding trials (see chapter 4 for details) where feeding deterrent chemical compounds have been isolated from endophyte-infected leaf tissue, and one assumes the same compound/s may be operative in deterring oviposition (through the act of excavating an egg cavity), then it seems unlikely that phytoalexins (as defined by Harborne and Ingham 1978) or the endophyte mycelium itself are responsible. However, these possibilities cannot as yet be completely ruled out. Chemical resistance rather than mechanical resistance of the endophyte mycelium itself is indicated by the results from ASW ovipositional preferences between Ariki + and Ruanui +. The Ariki + cultivar has a greater concentration of endophyte mycelium within leaf base tissue (an ELISA index of 5.754) than Ruanui + tissue (ELISA index of 5.114), yet weevils prefer to lay eggs in Ariki + (a mean number of 0.94 eggs laid per tiller in comparison to 0.91 eggs per tiller for Ruanui +). This anomaly could perhaps be explained by different levels of a deterrent chemical being produced between cultivars. With respect to feeding deterrents, Prestidge *et al.* (1985b) showed that this could quite likely be the case. When endophyte levels of three ryegrass cultivars (Droughtmaster, Nui, and Ellett) are all equal, there are still significantly different amounts of leaf tissue eaten. Thus it is not endophytic hyphae as such, but a plant-produced compound which is responsible for the deterrent effects. I have ignored this possible bias in my experiments and assumed that the same amount of deterrent compound is produced per quantity of hyphal tissue regardless of cultivar.

The relationship between endophyte incidence, as measured by ELISA indices, and ASW oviposition responses is shown graphically in Figure 14.

Figure 14

The Relationship Between Argentine Stem Weevil Oviposition in Various Ryegrass Cultivars and the Incidence of Endophyte.



The Regression Equation is :-

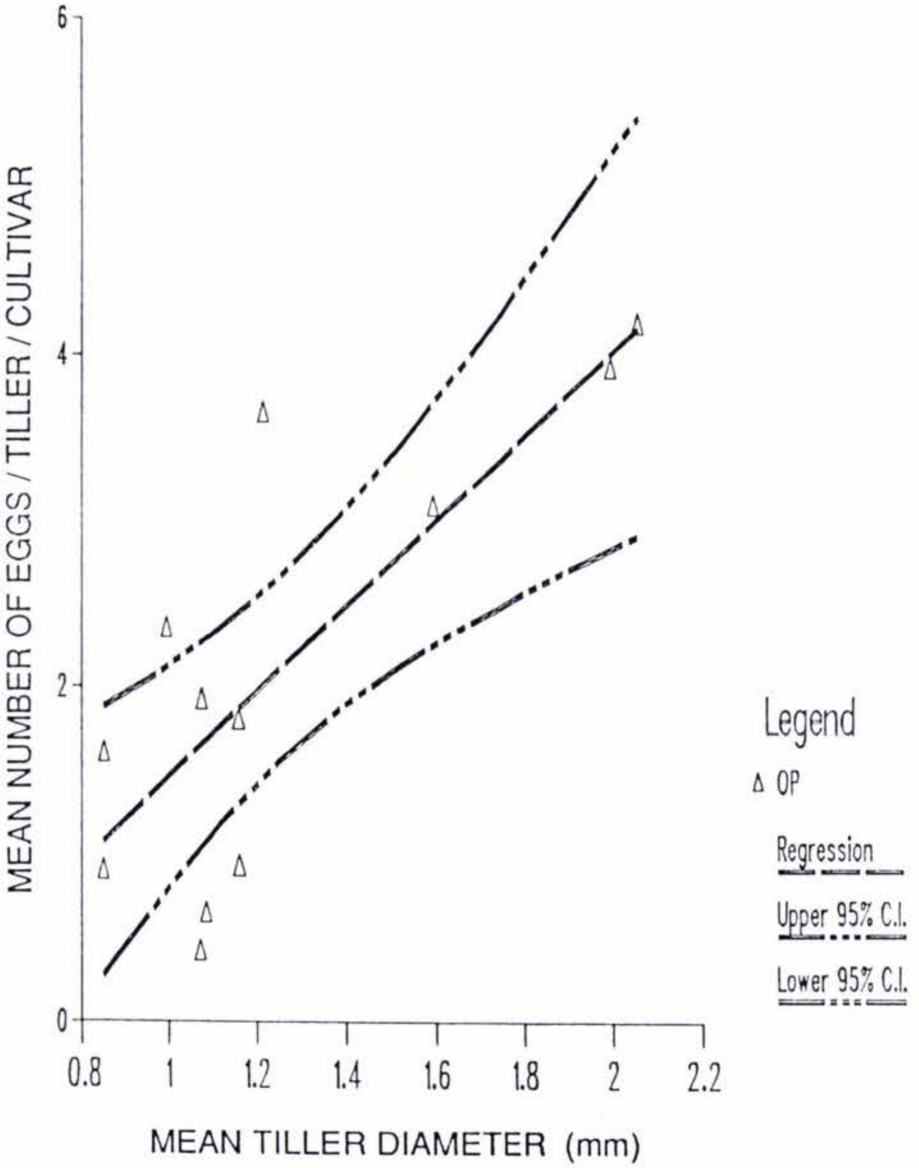
$Y = 3.16 - 0.382\,X$ $n = 12$, $R^2 = 0.57$, $P < 0.01$.

Tiller diameter also showed a consistent relationship to oviposition preference. Larger diameter tillers were preferred oviposition sites over smaller diameters, as shown by the regression analysis in Figure 15. These results are substantiated by the glass rod preference trials where the thicker diameter glass rods were preferred to the thinner ones, with 3 mm the optimum diameter (Fig. 13). It can be seen from Figure 12 that annual ryegrasses have thicker tillers than perennials, with hybrids intermediate. This further supports Goldson's suggestion (Goldson 1982) that susceptibility is related to genetic factors of short-rotation ryegrasses. Tiller thickness is associated with the type of leaf vernation - *L. multiflorum* ryegrasses are rolled, whereas *L. perenne* are folded. The folding of leaves not only makes them thinner, but tighter packed, and therefore tougher, (M. Forde pers. comm. 1986). This could mean that weevils experience greater difficulty excavating oviposition holes. Unfortunately this could not be substantiated with the penetrometer, but the results obtained in Chapter 4 concerning leaf lamina strengths suggest that the perennial ryegrasses are stronger than the annuals, and this is related to fibre content (Langer 1973, and Chapter 4). Examination of the fibre content of each cultivar (Fig. 11) shows that annuals have slightly less fibre within their sheath regions than perennials. It is therefore reasonable to suggest that oviposition preferences of ASW may be related to some extent to the ease with which the ovipositing weevil can penetrate the outer layers of the sheath material. In support of this Goldson (1982) found that timothy and cocksfoot were both favoured for oviposition compared to the harder tillers of tall fescue.

Possibly increased oviposition in larger diameter annual ryegrass tillers is associated with increased surface area available to the weevils. This is supported by the fact that annual ryegrasses grow up to 127 cm tall, while perennial ryegrasses are much shorter, growing only to 90 cm. Hence the amount of leaf sheath material available for oviposition is greater in annuals than perennials. Other authors (e.g. Pottinger 1977; Prestidge *et al.* 1985) have stated that mechanical resistance, such as hairy sheaths of Yorkshire fog, paspalum, and praire grass, account for the low susceptibility of these species to ASW. In the ryegrass cultivars examined (Table 6) no such external differences could be detected.

Figure 15

The Relationship Between Argentine Stem Weevil Oviposition and Mean Tiller Diameters of Various Ryegrass Cultivars.



The Regression Equation is :-

$Y = -1.11 - 2.57\ X$ $n = 12$, $R^2 = 0.62$, $P < 0.01$.

Observations of the behaviour of individual weevils on resistant and susceptible cultivars of ryegrass provided a wealth of information. For example, the time taken to reach a tiller by either sex was quite variable. This lends support to earlier conclusions (Chapter 3) where it is suggested that host-plant location is by chance. Also of note is the "exploratory" behaviour of weevils' when they encountered a tiller, when their antennae were either tapped on the tiller or waved in the air. The antennae of insects are considered the most important loci of olfactory and touch receptors (Dethier 1947), thus touching the surface with the antennae brings these receptors into contact with the plant. Similarly, the tarsal receptors are in contact with the tiller as the weevil grips the plant surface and the mouthpart receptors in contact with tissue during the process of excavating an egg cavity. Selection of a suitable plant may therefore be based primarily on touch and or taste. If a plant is identified as suitable for oviposition, further "selection" may occur via receptors on the ovipositor. These receptors and associated structures are considered in Chapter 6.

After behavioural tests were concluded and plants removed from the arena and inspected visually, two interesting features were observed. First, plugging of the oviposition hole with frass when the density of the weevils was increased, and second, the position of eggs laid on the resistant cultivar Ellett +.

Sealing oviposition holes with faecal pellets is common in many Curculionids. For example the alfalfa weevil (Milliron and MacCreary 1955; Hogg and Kingsley 1983), boll weevil (Mitchell and Cross 1969; Everett and Ray 1962), butternut curculio (Corneill and Wilson 1979), granary weevil (Kanaujia and Levinson 1981), kola nut weevil (Daramola 1976), sitka spruce weevil (Silver 1968), and the sunflower stem weevil (Charlet 1983) are all reported to do this. Three possible explanations are given in the literature. Sweetman (1929: in Manglitz and App, 1957) suggested that low relative humidity retards embryonic development of alfalfa weevil eggs. Thus sealing the oviposition hole with faeces might explain the insertion of eggs into stems and the sealing of punctures. A similar view is held by Daramola (1976) who believes plugging could prevent dehydration of the eggs; Barker *et al.* (1984c) noted that many ASW eggs desiccated in the field before hatching. A second hypothesis is that sealing prevents the eggs being attacked by natural enemies (Daramola 1976). Certainly in ASW's country of origin there are wasps which parasitise the eggs. Examples include *Potasson atomarius* (Brethes), [Hymenoptera: Mymaridae], and the less important *Sericophanes obscuricornis* Popp. (Miridae); and predators such as *Nabis punctipennis* Blanch. (Nabidae); and *Philonthus* sp. (Staphylinidae), (Ahmad 1977, 1978).

Sealing puncture holes may therefore reduce egg predation. The third possibility, proposed by Prokopy (1981), relates to epideictic or spacing pheromones.

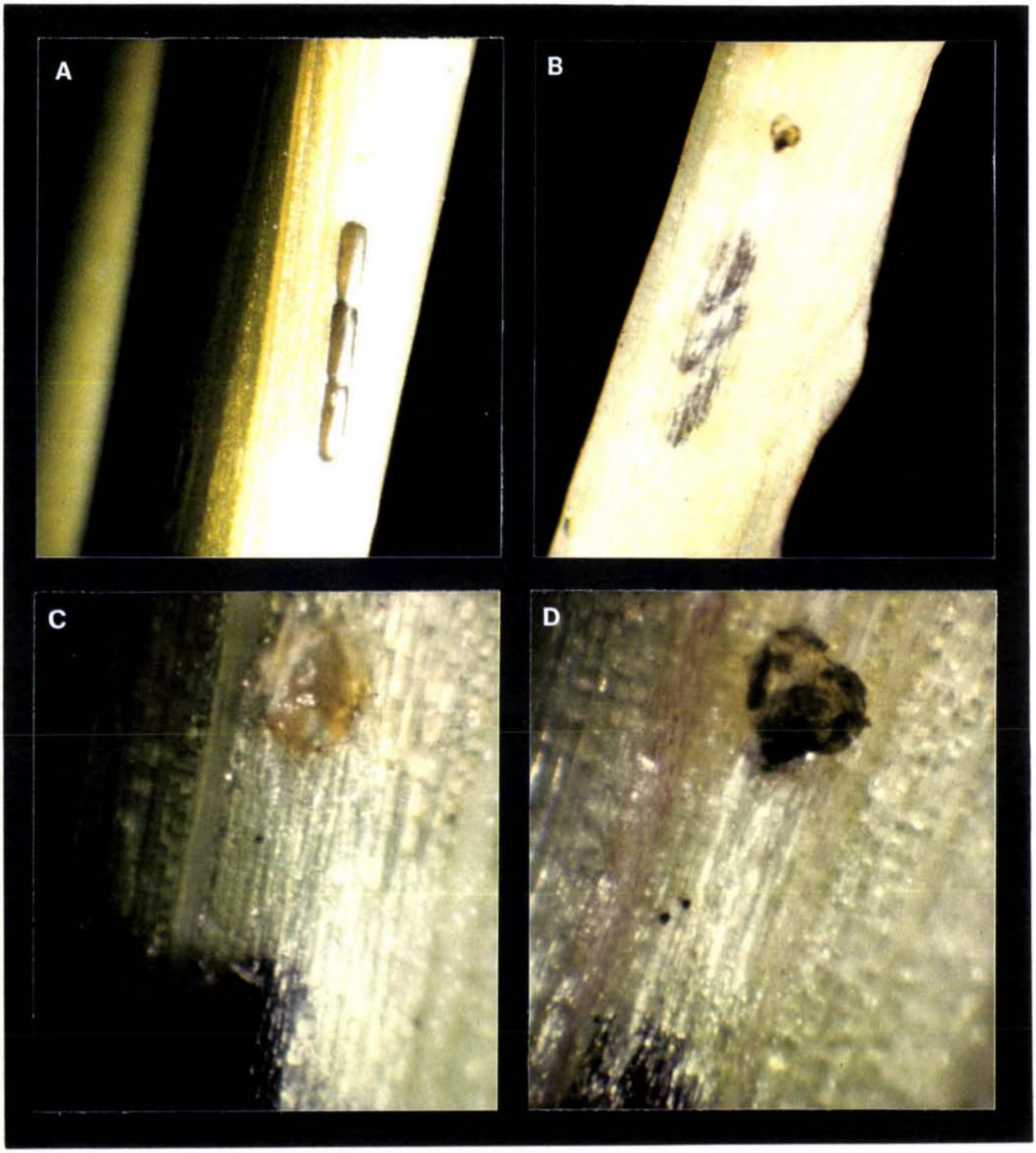
Females of many phytophagous insects avoid ovipositing on host-plants or parts of host-plant that already bear conspecific eggs. This behaviour produces nearly uniform dispersion of eggs amongst hosts and reduces intraspecific competition among larvae. It is especially characteristic of those insect species where the larval developmental site is confined to limited food resources (Prokopy 1981). Chemical spacing markers have been documented for the azuki bean weevil (Oshima *et al.* 1973; Wasserman 1981), while Mitchell and Cross (1969) observed that female boll weevils usually did not lay eggs in cotton squares that had already been punctured. Additionally Kozlowski *et al.* (1983) provide evidence of an epideictic pheromone in the cabbage seed weevil.

Oviposition holes were plugged in the present study only when the density of the adult ASW's were increased. Plugged oviposition punctures appear black, in contrast to unplugged and clear excavation holes (Plate 6). While not too much emphasis should be placed on this observation because of the very small sample size, it could indicate another example of an epideictic pheromone.

Prestidge and Van der Zijpp (1985) showed that stem weevil population survival (egg to adult) was strongly negatively correlated with initial egg density. Tests for density dependence were positive. At egg densities greater than about 1000/m^2, survival was consistently less than 10%. At egg densities less than 200/m^2, survival was near 100%, and thus there was reduced intraspecific competition between the larvae.

In further trials Prestidge *et al.* (1987) found that the mean number of eggs laid per oviposition was 1.8. The maximum percentage of ryegrass tillers with multiple oviposition (egg batches) was 3%, in either endophyte-free or endophyte-infected ryegrass cultivars. The maximum grass tillers infested with eggs never exceeded 27%, therefore an unused pool of oviposition sites remained. Increased egg density was due to more tillers being used for egg laying rather than more eggs being laid per tiller, which may occur once all available tillers had been utilised. This could be due to the adults detecting an epideictic pheromone and thus the presence of eggs or of larvae through their frass. Adult plugging would therefore deter further adult oviposition on these tillers. After hatching, frass is deposited behind the larva as it tunnels into the grass tiller. This perhaps reinforces the pheromone, as larval food and adult food are identical. It has been recorded that frass from feeding larvae

Plate 6



Differences between ASW oviposition punctures with two different densities of adults.

A. Oviposition puncture from one female. Three eggs are visible through the epidermis below the puncture. (Magnification X 13).

B. Oviposition puncture with five females present. The blackened area is frass which has been excreted into the excavation hole. (Magnification X 13).

C. and ***D***. Enlarged photographs of ***A***. and ***B***. respectively. (Magnification X 70).

of the cabbage looper deters oviposition by adult moths (Renwick and Radke 1980), and adult frass of the dry bean weevil on beans results in their avoidance by other females for oviposition (Szentsesi 1981). Whether or not the deterrent effect of frass on repeated oviposition of ASW is due to an epideictic pheromone is as yet uncertain, but offers future experimentation.

A second interesting feature of the behavioural tests is the position of egg deposition in the resistant cultivar, Ellett +. Eggs were laid less than 4 cm. away from the tip of the leaf lamina on every occasion, between the upper and lower epidermis. Of all factors which could account for this, the distribution of the fungal endophyte *Acremonium lolii* is the most likely. Results presented in Chapter 4 with respect to feeding between the tip and base of endophyte-infected plants (Table 9), show that the weevil can readily detect endophyte and hence deposit its eggs where endophyte is absent. Musgrave and Fletcher (1984) recorded an endophyte mycelium concentration in leaf lamina tissue of 2%, and this was concentrated towards the ligule (Musgrave pers. comm. 1985). It has often been observed that ASW feeding is restricted to leaf tips (Pottinger 1961a), also attributed to the lowered concentration of endophyte. If the deterrents for oviposition and feeding are identical or similar, then eggs should be laid where endophyte or endophytic compounds are absent, hence the leaf tips. However, laying of eggs within the lamina tissue is potentially mal-adaptive ecologically, as this tissue is the first to be consumed by larger grazing herbivores. In the confined space and limited choice of the test, plus a possible "ready" physiological state to oviposit, normal oviposition selection mechanisms may be overridden and weevils may oviposit in the "best" available position.

In summary :-

Oviposition preference of ASW is related to both tiller diameter and endophyte incidence (Figs. 14 and 15 above). Multiple regression analysis gives the best linear fit for this relationship as:

$$Y = 1.87\, X - 0.236\, Z + 0.486$$

where both X and Z are significant at $P < 0.01$. $n = 12$.

(Y = oviposition preference, X = tiller diameter, and Z = endophyte incidence). Tiller diameter and endophyte incidence account for 84% of the variability in oviposition preference.

CHAPTER 6

THE EXTERNAL RECEPTORS USED BY ARGENTINE STEM WEEVIL DURING HOST-PLANT SELECTION.

INTRODUCTION.

Work described in the preceding three chapters has been directed primarily towards laboratory bioassays associated with the three main phases of host-plant selection behaviour; orientation, feeding, and oviposition. However, before an understanding can be achieved of the mechanisms involved in regulating these three processes, knowledge of the structure and function of the insect's exterior sensory receptors (exteroreceptors) must be obtained (Payne *et al.* 1973; Alm and Hall 1986). Insect exteroreceptors are most numerous on the appendages of the head (antennae and mouthparts), thorax (legs and wings) and anal segments (cerci and genitalia) (Zacharuk 1985). Each of these areas was examined separately using Scanning Electron Microscopy (SEM) to identify the exteroreceptors of ASW. These exteroreceptors of ASW have been classified according to Zacharuk (1985), possible functions ascribed from the typology proposed by Altner (1977), and all compared with relevant Curculionid literature where possible.

The sensory exteroreceptors of most importance to an insect in regulating behaviour are those that receive mechanical energy generated by physical forces such as touch, pressure, or vibrations, (mechanoreceptors), and those that detect chemical stimuli, (chemoreceptors). The latter can be further subdivided into olfactory (smell) or gustatory (taste) receptors, depending on whether the detected chemical is in a liquid or gaseous state. Probably less important are the geo-, thermo-, and hygroreceptors.

It must be emphasised that though there are similarities in the external morphology of the sensilla found in this investigation and those studied externally and histologically by other investigators, experimentation must be carried out on specific sensilla to determine internal morphology through Transmission Electron Microscopy (TEM). Furthermore, before a specific function can be assigned to any given sensillum with any confidence, electrophysiological investigation must be carried out. Both TEM and electrophysiological experimentation were beyond the scope of this thesis, but the foundations have been developed, both in this chapter and in Chapter 3 respectively.

MATERIALS AND METHODS.

Weevils with their ovipositor exerted were prepared for scanning electron microscope (SEM) examination by placing entire specimens in modified Karnovsky fixative of 3% gluteraldehyde and 2% formaldehyde in 0.1 M phosphate buffer (pH 7.2). Specimens were then buffer washed and dehydrated in an acetone series before being critical point dried using liquid CO_2. Specimens were then glued to aluminium stubs with conductive silver paint and sputter coated with 20 nm of gold using a "Polaron" E5100 series "cool" sputter coater. Other specimens were air dried before gluing and sputter coating. Specimens were examined under either a Philips 505 SEM, or a Cambridge 250 "Mark III" SEM at 20 kV accelerating voltage.

RESULTS AND DISCUSSION.

Scanning electron microscope examination of ASW exteroreceptors revealed a varied array of sense organs. The different appendages bearing exteroreceptors will be discussed in turn.

Antennal Receptors.

Insect antennae are a pair of bilateral preoral appendages of the head. The basic three-part structure is uniform among insect groups. The basal part is a single segment termed the scape, which inserts in a membranous antennal socket on the cranium. The second part also consists of a single segment: the pedicel. The remaining distal part is the flagellum, and is the most variable. ASW has an elbowed antenna, with a seven-segmented flagellum (Plate 7A), the last segment of which is delineated into three regions by constriction bands, forming the antennal club (Plate 7A and 7B).

The primary function of the antennae is to bear exterosensilla, and to bilaterally extend these ahead of the body. This seems to be the sole function in the majority of insects (Schneider 1964). However, the distribution of sensilla on insect antennae is not uniform. The scape and pedicel primarily bear mechanosensilla of several types which function as extero- or proprioreceptors. They monitor the active movements of these segments and the passive deflections, vibrations or other movements of the flagellum at its base. The greatest number of antennal sensilla, and of different sensilla types, occur on the flagellum, with the highest proportion on the club itself. In ASW, the antennal club contains approximately 80% of all the sensilla. These are identified in more detail as follows:-

Sensilla trichodea (s.t.) type I occur primarily on the club and project outward and angle anteriorly (Plate 7B). These sensilla are blunt tipped and have faint longitudinal grooves, averaging 90µm in length (range 70 - 120µm), and are most likely a uniporous type of sensillum. Because of their great length, slender structure and outward projection, Mustaparta (1973) suggests a contact chemoreceptor, and/or mechanoreceptive function for this type of sensillum on the antennal club of the pine weevil, *Hylobius abietis*, a sensilla which is similar to s.t. type I found on the clover head weevil, *Hypera meles* (F.) (Smith *et al.* 1976); s.t. type II on the pecan weevil, *Curculio caryae* (Horn) (Hatfield *et al.* 1976), s.t. type I on the alfalfa weevil, *Hypera postica* (Gyllenhal) (Bland 1981), and the type V hairs on *Conotrachelus nenuphar*, the plum curculio (Alm and Hall 1986).

Sensilla trichodea type II (Plate 7C) are very common, longitudinally grooved slender receptors that are slightly curved or distinctly hooked at the pointed apex. They are found over the entire club, and are arranged in a general distribution over each segment. They range from 30 - 55µm in length. The sensilla point anteriorly and generally do not project outward at large angles as do s.t. type I. These sensilla are probably multiporous chemosensilla responsible for odour and/or water vapour perception. These sensilla trichodea type II are nearly identical in external appearance to the s.t. type I on *C. caryae* (Hatfield *et al.* 1976), similar to s.t. type II on *H. meles* (Smith *et al.* 1976), s.t. type II on *H. postica* (Bland 1981), and type III hairs on *C. nenuphar*. This sensillum also resembles a sensillum trichodeum described by Moeck (1968) on *Trypodendron lineatum* antenna, and by Payne *et al.* (1973) for several other species of Scolytid beetles. Moeck (1968) and Bordon and Wood (1966) suggested an olfactory function for this sensillum. However, on the antennal club of *H. abietis*, Mustaparta (1973) reported no dendrites in the lumen of a similar sensillum trichodeum, and concluded it had no olfactory function, later suggesting that they serve as mechanoreceptors or have merely a protective function (Mustaparta 1975). Because of the large numbers of these sensilla it seems unlikely that they all serve as guard hairs.

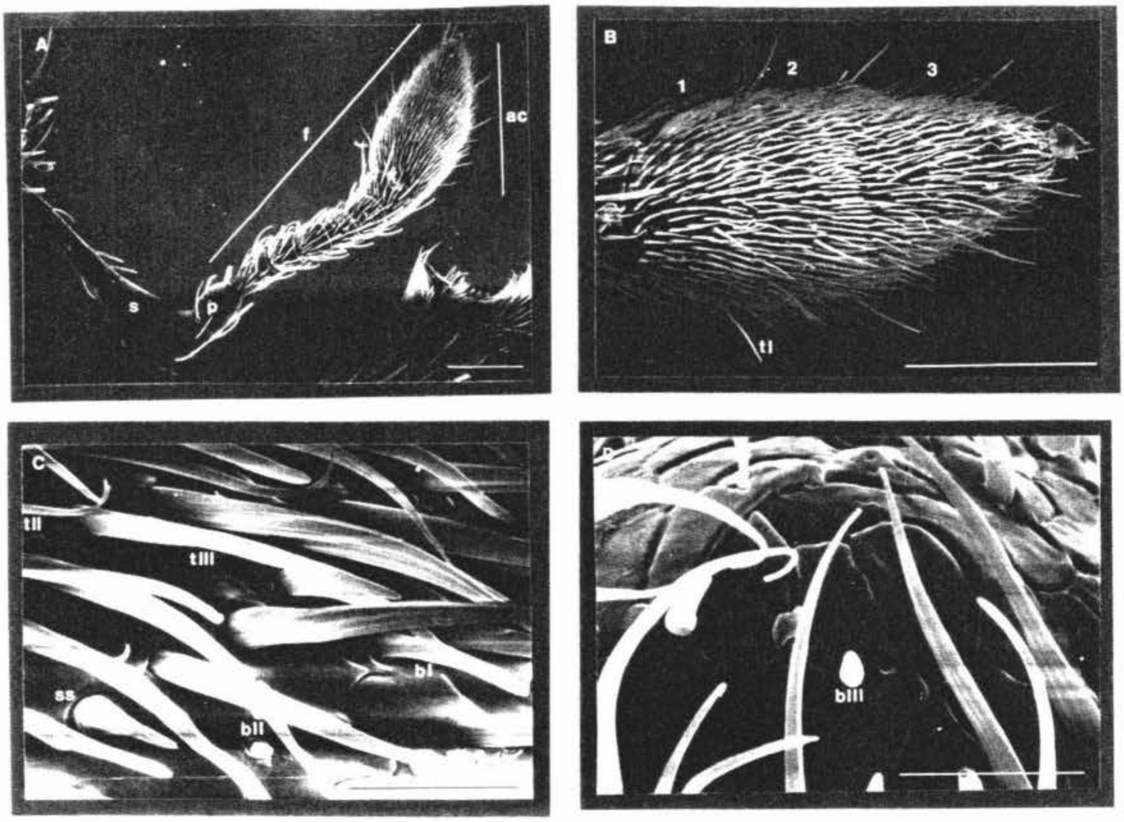


Plate 7

A) S.E.M. of the ASW antenna, showing the long scape (s), pedicel (p), and the flagellar segments (f) 1 - 10. The antennal club (ac) is formed by a fusion of the last four flagellar segments.

(Line indicates 0.1 mm). (Magnification X 156).

B) The last four segments of the flagellum which delineate the antennal club into three regions (1, 2, and 3). Also labelled is a long sensillum trichodea type I (t I).

(Line indicates 0.1 mm). (Magnification X 406).

C) High power S.E.M. between the club segments 2 and 3 showing sensilla trichodea type II and III (t II and t III); a sensillum styloconica (ss) and sensilla basiconica type I and II (b I and b II).

(Line indicates 10 μm). (Magnification X 3540).

D) The apex of the antennal club showing the cuticular pits (cp) and a sensillum basiconica type III (b III).

(Line indicates 10 μm). (Magnification X 4000).

Sensilla trichodea type III occur most frequently near the intersegmental lines of the antennal club (Plate 7C). The length of this sensillum ranges from 14 - 20µm. The tips of the sensilla are blunt with an apical depression. These sensilla thus appear to be uniporous chemosensilla.

Sensilla basiconica (s.b.) type I are short, blunt sensilla, slightly curved, occurring in rows primarily about the constriction bands. They are between 8 and 12µm long. They are the most common basiconic type (Plate 7C). Similar sensilla have been described on the antennae of weevils by Alm and Hall (1986), Bland (1981), Hatfield *et al.* (1976), Mustaparta (1973) and Smith *et al.* (1976). Thin sections of these sensilla taken by Alm and Hall (1986) revealed a multiporous, wall-pored form with two sense cells that branch distally, suggesting an olfactory nature.

A further sensilla basiconica (s.b. type II) was found within the constriction band delineating antennal segments 8 and 9 (Plate 7B). It is a small sensillum, only 1µm in length. Hatfield *et al.* (1976) found this type of sensillum on the antennae of the Pecan weevil, but mentioned that they seem to be absent or uncommon in Curculionids. Norris and Chu (1974) reported structures very similar on the antennae of *Periplaneta americana*.

Sensilla basiconica type III (s.b. III). The blunt club apex contains two sensilla basiconica type III which are 2 - 4µm long (Plate 7D). Each sit in a shallow crater. They appear to have a terminal pore. They are very similar in shape and position to s.b. type IV (Bland 1981), s.b. type III of *H. meles* (Smith *et al.* 1976), type IV of *C. caryae* (Hatfield *et al.* 1976), and to the pegs in *C. nenuphar* (Alm and Hall 1986).

Sensilla styloconica (s.s.). One slender s.s. with distal grooves was observed in the s.b. type III constriction band, very close to the s.b. type II sensilla (Plate 7C). The basal third of the s.s. is expanded and the sac-like base has a distal depression. The sensillum is 5µm in length, and 1µm in basal diameter. This sensillum is very similar in appearance and location to the more common s.s. of *H. meles* (Smith *et al.* 1976) and *H. postica* (Bland 1981). Dickens and Payne (1978) referred to them as fluted sensilla in the southern pine beetle, and noted that these sparse sensilla occurred singly in openings in the s.b. bands. McIver (1974) studied similar grooved pegs on the antennae of the mosquito, *Aedes aegypti* (L.) and found them to function as olfactory receptors. Further references to insects with these grooved pegs are given by Harbach and Larsen (1977) and Zacharuk (1980). The former authors indicate that the sensilla may have a hygroreceptive function. However, the s.s. on the ASW antennae are not numerous enough to be the major hygroreceptors.

Shallow-rimmed craters occur on the club apex. No function has been suggested for these formations. Craters of a similar nature occur in *H. postica* (Bland 1981).

Cuticular pits dot the surface of the antenna and are generally associated with sensilla. They are round to oval with very low rims (Plate 7D). No interior sensilla were observed in these pits. Bland (1981) suggests they are probably epidermal gland ducts in *H. postica*. Alm and Hall (1986) also observed these cuticular openings but did not ascribe any function to them.

The antennae of males and females, including dorsal and ventral surfaces, were compared for differences in numbers and types of sensilla, but no consistent differences were observed.

Mouthpart Sensilla.

The mouthparts of insects are the cephalic parts and appendages that are involved in feeding and food ingestion. Usually these are the labrum, mandibles, maxillae, labium and hypopharynx. In Curculionids, however, the labrum is absent (Britton 1973) (Plate 8A). They are structurally modified to various extents among insects primarily in relation to their mode of feeding. From a sensory standpoint, while the antennae are most often viewed as the insect's feelers and organs of smell, some of the mouthparts are considered as its organs of taste, with various types of mechanosensilla to monitor external forces and the positions and movements of the parts themselves (Zacharuk 1985).

The paired mandibles are more or less triangular structures, very heavily sclerotised, and with muscles inserted in their base. They are usually flattened dorsoventrally and have a convex outer margin. The inner margin is variably toothed to the apex. The mandibular sensory fields and their innervation are basically similar among insect forms. There are two or more hairs on the outer surface, and several to many campaniforms on the outer surface (Zacharuk 1985).

The maxillae of insects are a pair of head appendages that are positioned laterally or ventrolaterally to the mouth. There are two basal articulating segments, the proximal cardo and the distal stipes. Two lobes are attached to the stipes distally, the mesal lacinia and the outer galea. The most conspicuous appendage of the maxilla is the palp, which is attached to the side of the stipes. The palp of an insect maxilla is considered to be primarily a sensory appendage. Mechanosensilla are usually distributed along the shaft, with surface hair and internal scolopidia in the intersegmental joint areas, and campaniform organs and vari-sized tactile hairs scattered sparsely to densely over the surface of the segments. ASW is no exception (Plate 8B). The chemosensilla are primarily or wholly concentrated at or near the tip of the terminal segment (Plates 8B, 8C and 8D). Some of these also have mechanosensitivity. The terminal segment of the three-segmented palpus has either four or five longitudinal furrows on the ventro-lateral portion of the basal half (Plate 8B). These aporous sensilla range from 13 - 23μm in length on a single segment. Their varied forms and fields in adult Coleoptera are presented by Honomichl (1980). Bland (1984) states that they are probably placoid sensilla, although the majority of authors (Bellamy and Zacharuk 1976; Zacharuk *et al.* 1977; Doane and Klinger 1978; Whitehead 1981; and Hallberg 1982) state that they are probably digitiform sensilla. Zacharuk (pers. comm. 1985) considers that they are probably digitiform sensilla in ASW. This type of structure has been shown to be mechanosensitive by Zacharuk *et al.* (1977), but Honomichl and Guse (1981) believe that this sensillum is sensitive to stimuli other than chemical or mechanical. Schoonhoven (pers. comm. 1985) says they probably represent mechanoreceptors in ASW.

The tip of the terminal maxillary palp segment bears 21 sensilla basiconica (Plate 8B, 8C and 8D). Apical pores were resolved on most of these sensilla, and it seems likely that all have apical pores. The arrangement and shape of these sensilla varies, but generally there are 6 to 8 large central ones up to 5μm in height, and a ring of shorter sensilla ranging down to 2μm. One subapical sensillum occurs in each of the two depressions on the inner face of the terminal segment (Plate 8C). A comparison between Plates 8B and 8D reveals that the entire cluster of sensilla may be depressed into the tip of the palp. While the other cuticular sensilla of the maxillary palp are on sclerotised cuticle and occasionally on intersegmental membrane, the terminal sensilla clusters are invariably on patches of flexible cuticle as described by Altner (1975). This allows individual sensilla to yield inwardly under pressure.

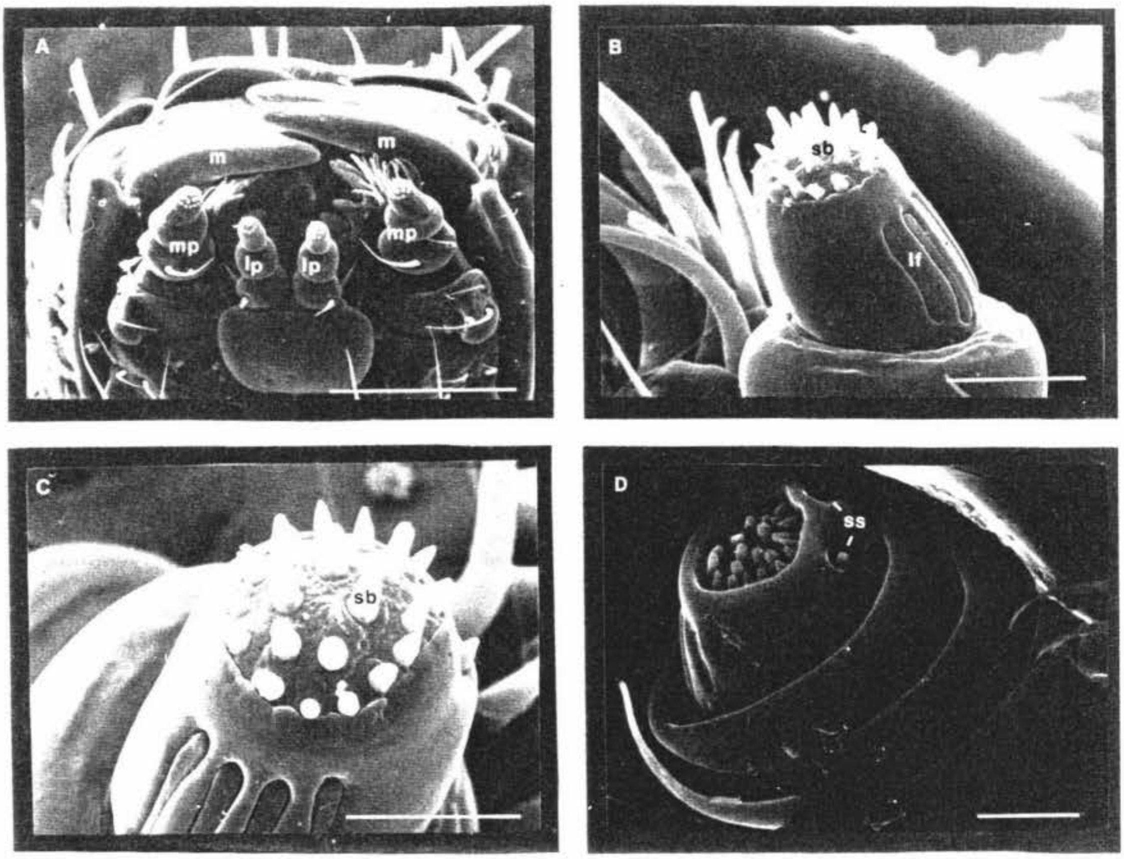


Plate 8

A) Ventral view of ASW mouthparts showing the paired mandibles (m), maxillary palps (mp), and labial palps (lp).

(Line indicates 100 μm). (Magnification X 300).

B) A maxillary palp showing sensilla basiconica (sb) on the apex, and longitudinal furrows (lf).

(Line indicates 10 μm). (Magnification X 2100).

C) Higher power of maxillary palp, showing 2 subapical sensilla (ss).

(Line indicates 10 μm). (Magnification X 3200).

D) Retracted position of the maxillary palp.

(Line indicates 10 μm). (Magnification X 2000).

The cluster of basiconic pegs at the tip are probably all uniporous taste receptors with a mechano-sensitive dendrite present in most (Zacharuk pers. comm. 1985). These uniporous sensilla generally sense by contact or gustation, but they can also respond to odours (Schoonhoven and Dethier 1966; Dethier 1972; Blaney 1977; and Klein 1981). They most often occur on insect appendages used in sensing by contact: hairs on tarsi (Hansen and Heumann (1971); Ma and Schoonhoven (1973; and Stádler (1978)); hairs and pegs on the tip of the labellum (Dethier 1971; and Stöckow 1970) and on the terminal segments of antennae (Harbach and Larsen 1976 and 1977; Zacharuk and Blue 1971), maxillary and labial palps (Bareth and Juberthie-Jupeau 1977; Bellamy 1973; Ma 1972; Mitchell and Schoonhoven 1974; Stádler and Hanson 1975; Devitt and Smith 1982), galea (Mitchell and Schoonhoven 1974) and cerci (Schmidt and Gnatzy 1972); and pegs, papillae, or plates on the ovipositor (Hooper *et al.* 1972, Rice 1976) and in the buccal cavity itself (Cook 1972; Moulins 1971; and Wensler and Filshie 1969).

The labium consists of a pair of appendages that are serially homologous with the maxillae, but which are fused together medially to various degrees in the different insect forms. The labium consists of a basal mentum, two pairs of anterior lobes and a pair of lateral appendages, the palps (Plate 9A). The palps consist of one to four segments and are primarily sensory in function. In ASW the labial palps are three-segmented (Plate 9B). The apex of the third segment bears 10 sensilla basiconica ranging from 3 to 6.5µm in height (Plates 9C and 9D). Most were observed to have small apical pores, with one fluted by finger-like projections (Plate 9D). These sensilla are similar to sensilla basiconica on the maxillary palp terminal segment, and have similar functions. A few tactile hairs (sensilla trichodea) and cuticular pits can also be observed on the lower two segments (Plate 9D). No internal sensilla were observed within these pits with the SEM and could thus represent epidermal gland ducts as in the antennae.

Within the buccal cavity itself are a wide range of sensilla. Groups of slender sensilla trichodea and finger-like sensilla basiconica of various lengths and shapes project toward the centre of the oral opening. These could be mechano- or chemo-sensilla, as could the larger horn-like sensilla, but as no pores are visible then only TEM is likely to determine their function. The number and variety of sensilla indicate that considerable food evaluation may occur in the buccal cavity after the initial sampling by maxillary and labial palps, and cutting by the mandibles.

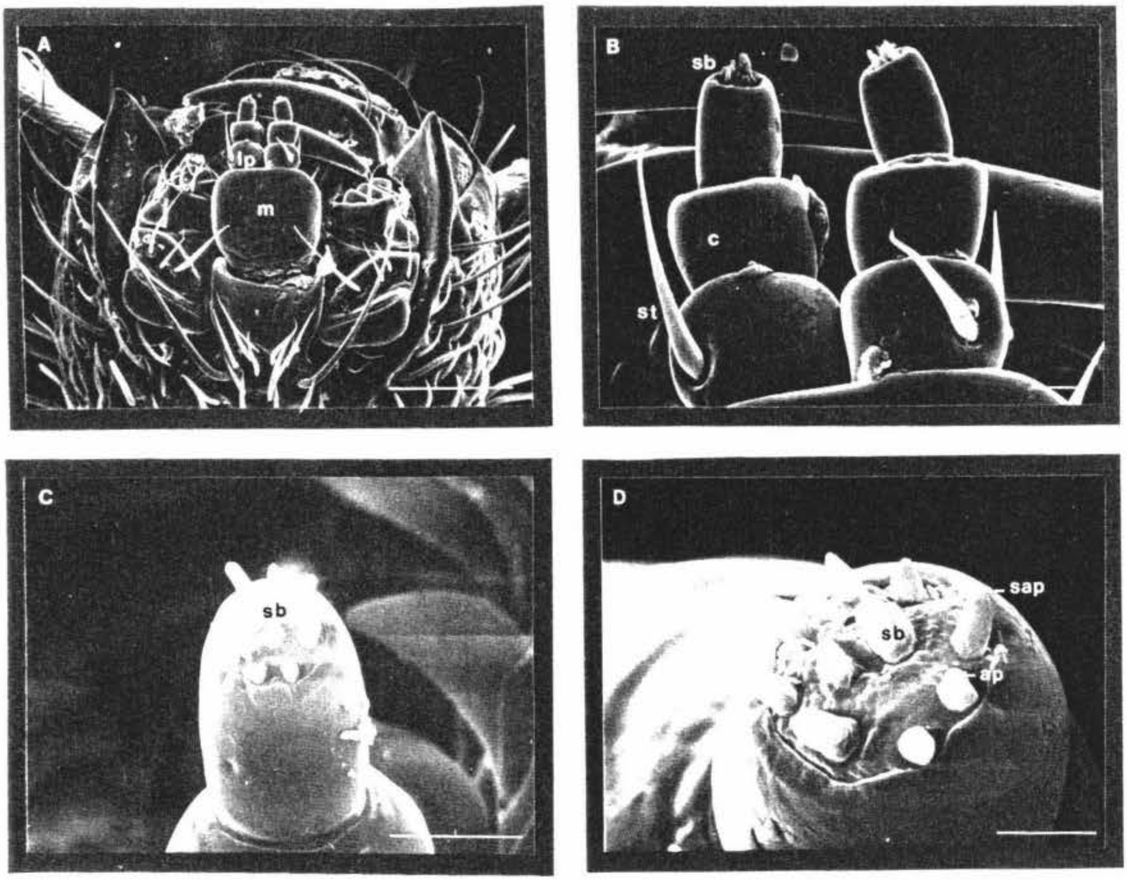


Plate 9

A) Ventral view of ASW mouthparts showing the labium which consists of the mentum (m) and a pair of labial palps (lp).

(Line indicates 0.1 mm). (Magnification X 240).

B) The three - segmented labial palps showing trichoid sensilla (st), cuticular pits (c) and also the basiconic pegs on the terminal segment (sb).

(Line indicates 10 μm). (Magnification X 1150).

C) Terminal segment of the labial palp showing 10 sensilla basiconica (sb).

(Line indicates 10 μm). (Magnification X 2800).

D) Higher power of ***C*** and the blunt tipped pegs (sb). Most contain an apical pore (ap), one with finger-like projections closed around it, a slitted apical pore (sap).

(Line indicates 4 μm). (Magnification X 5000).

Tarsal Receptors.

The legs of insects are appendages of the thorax adapted primarily for walking and running, although in specific groups they can be modified for other functions (grasping prey, swimming, burrowing). To monitor these functions they have extensive fields of diverse mechanosensilla on the surface. In a few insects, the legs have been shown to house organs of taste. These are concentrated toward the tarsi. Hairs or pegs with a structure and innervation typical of contact chemosensilla have been noted in a few species, but are undoubtedly more universal in occurrence (Zacharuk 1985). There is apparently no literature pertaining to Curculionid leg receptors, although ASW possesses many. As can be seen in Plate 10A, the four tarsomeres, collectively called the tarsus, possess numerous trichoid sensilla which presumably are mechanoreceptors (Bland pers. comm. 1986). Also, on the distal end of the tibial section of the leg, arranged mainly in two rows, are numerous strikingly grooved pegs, (Plate 10B). Magnified in Plate 10C these appear to have a distal pore (or atleast a concavity) suggesting a uniporous gustatory function. At the apex of the tarsus is the pretarsus which has paired claws and a centrally placed arolium. Arising from this structure is a large sensillum trichodea, as are the sensilla in the surroundings (Plate 10D), which suggest possible mechanoreceptor and/or contact chemoreceptive functions because of their size.

Receptors on the Ovipositor.

The genitalia of insects comprise of one or more modified terminal abdominal appendages. The ASW ovipositor has relatively few exteroreceptors. When the ovipositor is exerted (Plate 11A), the eighth tergite and sternite (the spiculum ventrale) spread apart and the seventh and eighth tergites extend beyond the posterior edge of the elytra. The annulated foretube (Plate 11D) and smooth hindtube have a pair of elongated chitinous plates (baculi) which run from the mid-fold (through which access to the vagina is gained when the ovipositor is in its retracted position) to the coxites at the apex. On each of the coxites there is a small cylindrical stylus which bears several receptors. The external aperture of the reproductive system opens between them.

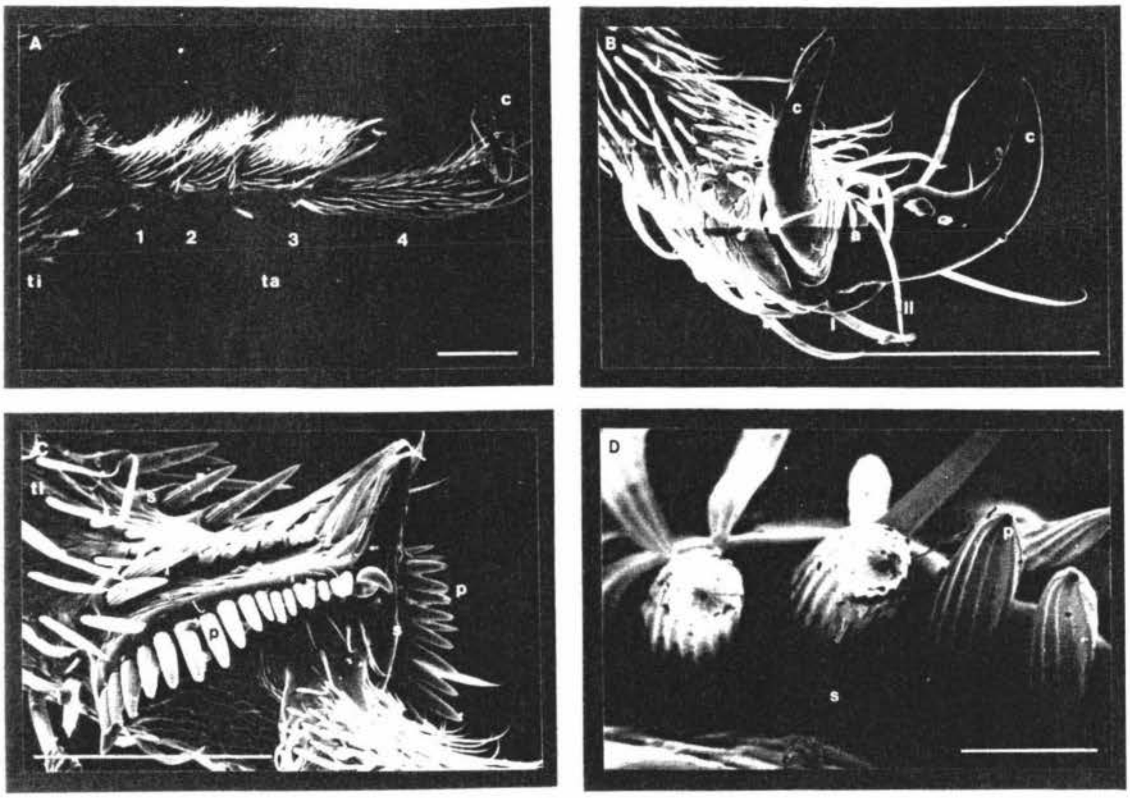


Plate 10

A) SEM of ASW foreleg showing the distal end of the tibia (ti), the four tarsomeres labelled 1 to 4 which make up the tarsus (ta), and at the apex the pretarsus consisting of the paired claws (c).

(Line indicates 0.1 mm). (Magnification X 178).

B) The pretarsus showing the paired claws (c), centrally placed arolium (a), and sensilla trichodea type I (I) and II (II).

(Line indicates 0.1 mm). (Magnification X 573).

C) Higher magnification of the grooved pegs showing the distal pore (p) and the socketed regions (s).

(Line indicates 10 μm). (Magnification X 2980).

D) Distal portion of the tibia (ti) showing the strikingly grooved pegs (p) which appear moveable in sockets (s).

(Line indicates 0.1 mm). (Magnification X 526).

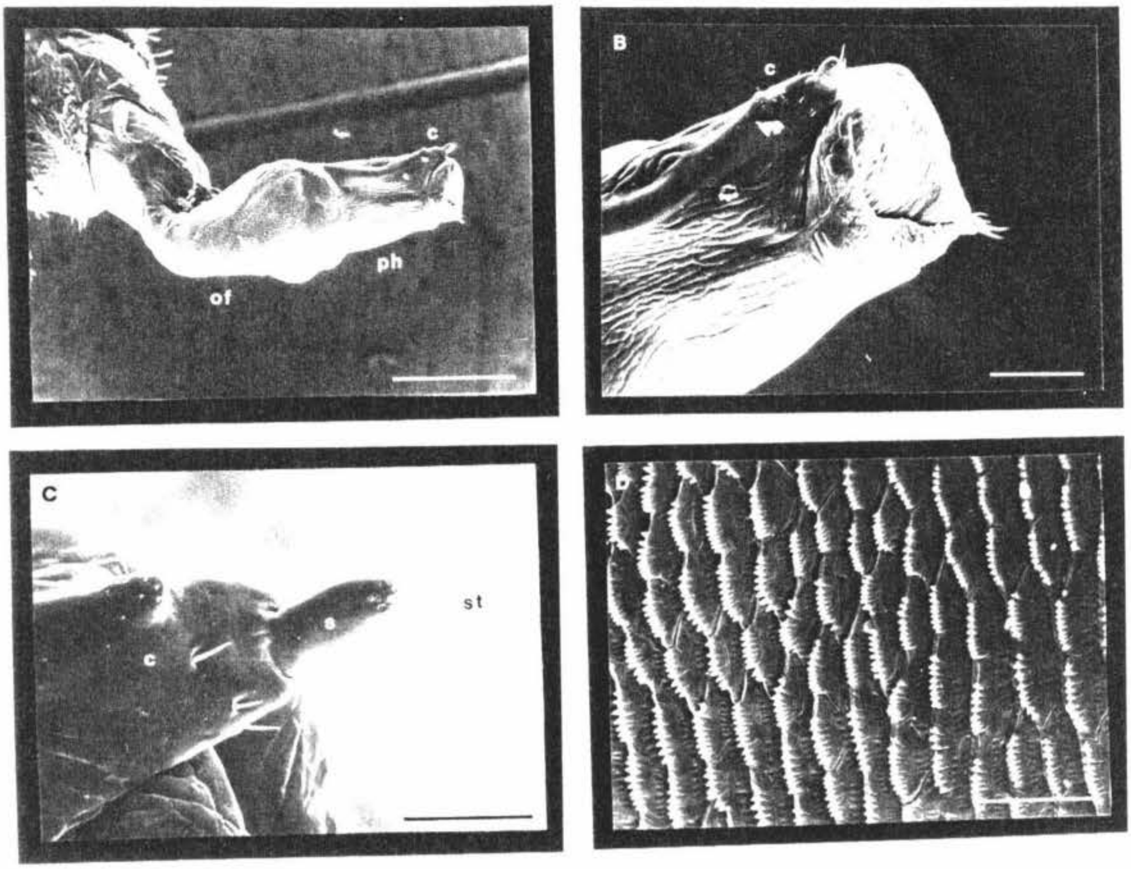


Plate 11

A) Fully extended ovipositor of ASW, showing the ornamented foretube (of), protruded hindtube (ph), and the coxite (c) which bears a small cylindrical stylus.

(Line indicates 400 μm). (approx Magnification X 7).

B) The external aperture of the reproductive system, with one of the paired coxites (c) labelled.

(Line indicates 100 μm). (approx Magnification X 200).

C) Enlarged SEM of the coxite (c), clearly showing the stylus (s) and four long setae or sensilla trichodea. Also visible are the s.b., numerous pits, and the domed sensilla around the s.t.

(Line indicates 40 μm). (approx Magnification X 700).

D) Portion of the ornamented foretube.

(Line indicates 20 μm). (approx Magnification X 1300).

In the literature studied, there is no mention of Curculionid styli being classified into sensilla types. They have only been called setae (Tanner 1927; Bissel 1937; Burke 1957; and Goldson 1979a). Of these setae, the four long sensilla trichodea (Plate 11B), probably represent mechanoreceptors which perceive the textural conditions of the oviposition substrate, and thus the sensory information they receive may play a role in ovipositional behaviour. They may also constantly signal the position of the sides of the excavated tunnel in which the eggs are being laid, as in the blowfly *Phormia regina* (Wallis 1962).

Clearly visible in Plate 11C are sensilla styloconica (domes or pegs). Usually such domes are assumed or proven mechano/chemosensitive (Zacharuk 1985). Wallis (1962) indicates that similar pegs on the ovipositor of the blowfly *Phormia regina* are olfactory receptors which are important in regulating oviposition. Their function is not known for ASW. Also visible in Plate 11C are numerous cuticular pits which are possible s. ampullacea, and/or s. campaniformia, but again, without TEM, correct identification is impossible.

The basic cuticular forms described above have been classified according to the list of Zacharuk (1985). These were identified on the basis of the form of their cuticular parts, i.e. purely on external morphology. This in itself gives very little insight into their functional morphology or modality. However, the following reviews give additional details for most of these types and interpretations of subtypes: Slifer (1961, 1970), Ivanov (1969), Sinoir (1969), McIver (1975), Altner (1977), and Zacharuk (1980).

From the scanning electron microphotographs there is no doubt whether the sensory cuticle of a sensillum projects far out, little, or is flat on the surface, which is significant in determining what stimuli it will be exposed to, or trap, and conduct to its transducer mechanism. To this extent a generalised typing into one of the categories used in this thesis is informative. However, whether a sensory projection is a peg, cone or cupola is all very much subject to individual interpretation. It is therefore imperative that comparative morphometric data be provided for the cuticular parts of the sensilla under study to eliminate such individual bias, as proposed by Mayer *et al.* (1981). Nevertheless, from the SEM study described above, especially from inferences with respect to antennal sensilla from other Curculionids, it is reasonable to assume that the numerous sensory receptors enable ASW to detect all the major sensory modalities. All appendages bear exteroreceptors, so all may potentially play a part in host-plant selection processes. However, as already stated, without the TEM and electrophysiological corroboration, their role in the three main processes of orientation, feeding, and oviposition cannot be separated.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

Three separate chapters have been devoted to the three behavioural responses of ASW to potential host-plants, namely orientation, feeding and oviposition. The results of experimentation incorporating these three entities have been thoroughly reviewed and discussed in detail at the completion of each chapter, as have plant characters possibly influencing these behaviours. This final chapter is an attempt to integrate information relative to these three responses and the plant characters, and to discuss some implications for managerial strategies for ASW.

Orientation, as stated in Chapter 3, is exceptionally difficult to investigate in the laboratory. Testing for visual discrimination between distinctly coloured nutrient agar food plugs produced no significant results, and hence this may indicate that ASW does not visually discriminate between susceptible and resistant host-plants in the field where colour differences are relatively subtle. However, it is possible that ASW needs to be in flight before it reacts to colours as Pottinger (1966) stated, but even if this does occur, then it is unlikely to offer specific orientational cues due to the subtle colour variations between plant species. Furthermore, no significant flight behaviour has been observed in the North Island (Gaynor pers. comm. 1985) where the present study was undertaken. It may be that ASW walks from one site to another in search of potential host plants relying on close range orientational cues. There is no mention in the literature of such behaviour for ASW, but there are many examples for other weevils (Danthanarayana 1970; Fischer and O'Keefe 1979; Underhill *et al.* 1955; Prestcott and Newton 1963; and Pausch *et al.* 1980). Mark and recapture experiments would possibly clarify this aspect of dispersion for ASW in the North Island.

The olfactory sense of ASW was also examined experimentally in the present study by observing the weevils' responses in an olfactometer towards odours of a susceptible and/or resistant ryegrass cultivar. No significant responses were obtained, which may indicate that ASW does not orientate to host-plants through the olfactory sense. Identification of the volatile chemicals emitted by the susceptible and resistant cultivar showed only the presence of "green leaf" volatiles, with nothing specific to ryegrasses which could act as an "attractant" for ASW. However, the foundation for future experimentation has been laid with the identification of these leaf volatiles which should be tested in association with the sensilla identified in Chapter 6 in an electrophysiological study, coupled with a TEM investigation.

The implementation of control measures to disrupt plant orientational cues (with the limited information available at this stage) would be too costly considering the extent of ASW hosts throughout New Zealand (see Table 1). Further complicated control measures with associated high costs must also be accrued if the explanation of "apparency" I have offered best explains ASW orientational responses (Chapter 3). The only method is to render the plants more "un-apparent", and again with the vast expanse of ASW hosts, the extent to which a "camouflage" approach can be implemented is not practical. However, with the very long list of variables attendant in understanding and interpreting orientation behaviour of ASW, it is perhaps not pertinent to consider "how" ASW initially selects its host-plants and infests New Zealand pastures, (especially as ASW is considered to infest virtually every improved pasture within New Zealand (Pottinger 1985)), but more important to recognise that ASW "has" selected the New Zealand pasture grasses as its host and to act upon this fact in the implementation of suitable control measures. It is thus the insect responses to the plants in relation to feeding and oviposition, the two parameters in which the insect is in intimate contact with the host-plant, which now become more relevant.

From Chapters 4 and 5 it may be concluded that ryegrass susceptibility or resistance to ASW oviposition and feeding correspond (in part) to the presence or absence of an endophytic fungus within the ryegrass tissue (*Acremonium lolii* Latch, Christensen and Samuels (Latch *et al.* 1984)). This is the more common of two endophytes isolated from perennial ryegrasses. The other is a *Gliocladium*-like species (Latch *et al.* 1984), which ASW actually prefers to feed upon (Gaynor *et al.* 1983). The results from the present study have shown that ryegrass plants with a high concentration of *A. lolii* endophyte within their tissues (determined by Enzyme-linked Immunosorbent Assays (ELISA), suffer proportionally lower amounts of feeding damage and oviposition. Conversely, tissue with a low endophyte concentration suffers a proportionally high amount of damage through both feeding and oviposition. ASW feeding is in fact selective enough to distinguish between different concentrations of endophyte mycelium between regions within a tiller. Similar associations have been demonstrated by Mortimer *et al.* (1982); Mortimer and di Menna (1983 and 1985); Prestidge *et al.* (1982); Gaynor and Hunt (1983); Gaynor *et al.* (1983); and Barker *et al.* (1983, 1984a, b, c, and 1986).

Insect resistance from grass/endophyte associations has now been observed for numerous other insect species. These include the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), Clay *et al.* (1985a and b), Hardy *et al.* (1985 and 1986); Southern armyworm, *S. eridania*, Ahmad *et al.* (1987); grey/brown cutworm, *Graphania mutans* (Lepidoptera: Noctuidae), McGee (pers. comm. 1987); bluegrass billbug, *Sphenophorus parvulus*, (Coleoptera: Curculionidae), Ahmad and Funk (1983) and Ahmad *et al.* (1985); sod webworms, *Crambus* spp. (Lepidoptera: Pyralidae), Funk *et al.* (1983); the large milkweed bug, *Oncopeltus fasciatus*, and greenbug, *Schizaphis graminum*, Siegal *et al.* (1985); corn leaf aphid, *Rhopalosiphum maidis*, Johnson *et al.* (1985); cereal aphid, *Rhopalosiphum padi*, Latch *et al.* (1985b); house crickets, *Acheta domesticus*, black beetle, *Heteronchus arator*, Ahmad *et al.* (1985); and pasture mealybug, *Pseudantonina poae*, Gaynor and Rowan (1986).

It is clear that endophyte infection produces some form of resistance to ASW. However, as it is now possible to artificially infect seedlings of ryegrasses with a range of endophytic fungi (Latch and Christensen 1985), the desirability or otherwise of such an infection must be carefully considered.

ASW resistance to endophyte-infected plants has major consequences for ryegrass persistence and production. In field situations, endophyte infections probably account for differences in pasture production of up to 30% between ryegrass cultivars. This has been observed in the Waikato (Barker *et al.* 1984a), Wairarapa and Manawatu (Kain *et al.* 1977), Hawkes Bay (Kain *et al.* 1982b) and Canterbury (Goldson and Trought 1980). Argentine stem weevil selectively attacks endophyte-free ryegrasses to the extent that Prestidge *et al.* (1984a) found endophyte levels increased in pastures by 25 - 400% per annum. A more recent survey of 40 *L. perenne* pastures by Prestidge *et al.* (1985c) showed a decline in ryegrass content from 90-95% in new sown swards to 35% within two to three years. In four to six year old pasture nearly all surviving ryegrass had endophyte infections, indicating the heavy selection pressure by ASW and emphasising the monetary savings from initially sowing endophyte-infected seed. According to P. Stewart (1986), "300,000 ha of land is regrassed in New Zealand each year costing farmers about $40 million per annum, thus high endophyte pastures could save farmers about $1 million annually."

Almost all summer persisting perennial ryegrass cultivars in New Zealand have now been found to contain *Lolium* endophyte (Latch and Christensen 1982; Scott 1983; Stewart pers. comm. 1986). This persistence of high endophyte pastures could well lead to a larger advantage in per hectare animal production if correctly managed (Kerr 1987), as endophyte-infected ryegrass pastures contribute to increased summer and autumn ryegrass yield. This is important in grassland farming as it ensures adequate winter/early spring feed and maintenance of desirable clover/ryegrass balances (Kain *et al.* 1982b). Without endophyte, most of New Zealand's 8 million ha of improved *L. perenne*/white clover based pastures would lose most of their *L. perenne* component within 2-5 years (Barker *et al.* 1981; Prestidge *et al.* 1985c). These clover dominant pastures may increase the likelihood of bloat the following spring (Batten 1964; Goldson and Trought 1980). Similar loss of ryegrass can result in weed growth, which may necessitate costly control procedures and re-sowing, plus the possibility of lack of pasture recovery in autumn leading to winter grazing shortages for livestock.

The endophytes themselves are not pathogenic to their hosts (Neill 1941); the fungi grow intercellularly without penetrating or apparently damaging the host cells (Siegal *et al.* 1985). This is a mutualistic relationship. The fungus benefits by being protected within the plant and by dissemination through the seed, while receiving nutrients from the ryegrass. The plants' benefits are more complex, involving increased tolerance to stress (characterised as resistance by the plant to insect attack), limiting overgrazing by herbivores, and by enhanced growth (Siegel *et al.* 1985). Nui plants infected with *A. lolii* showed significant increases in total leaf area, tiller numbers and growth of leaves, pseudostems and roots, plus yielded 38% more total dry matter than uninfected plants in a controlled environment room (Latch *et al.* 1985a).

The endophyte mycelium occurs intercellularly in all above-ground parts of infected perennial ryegrass. Infected seed germinates to produce seedlings infected with mycelium and this is the only way in which the fungus is spread (Neill 1940). The mycelium invariably dies if seed is stored for more than 18 months at ambient temperatures (Neill 1940), yet can remain viable if stored at low temperatures (0°C to 5°C) for up to seven years (Latch and Christensen 1982), or in a low humidity environment (< 60% RH) (Rolston *et al.* 1986). In addition, it is not necessary for the endophyte within the seed to be viable to confer seedling resistance to ASW (Stewart 1986). Stewart suggests this could be due to the translocation to the seedling of a remnant chemical associated with endophyte in the seed which is diluted or metabolised as the plant develops. These factors may explain the variable results from ASW feeding trials and why ryegrass breeding results, especially those relating to persistence and herbage regrowth, have often been spurious and non-repeatable (Harvey 1985).

There are, however, some undesirable effects of endophyte infection. Endophyte-infected ryegrass is less palatable to stock and may subsequently affect their growth and reproduction (Fletcher 1983; Fletcher and Barrell 1984). Of even more importance is the animal health problem "ryegrass staggers", a serious neurological disorder of grazing livestock in New Zealand (Cunningham and Hartley 1959). A very strong association was found between the presence and extent of *A. lolii* endophyte in ryegrass, and the incidence and severity of ryegrass staggers in sheep grazing these plots (Fletcher and Harvey 1981; Mortimer *et al.* 1982 and 1984; and Fletcher 1983). The effects of ryegrass staggers are mainly observed in summer and early autumn, a period coincident with peak endophyte growth and summer droughts. Restricted pasture growth forces stock to graze lower and ingest tiller bases where most of the endophyte infection is found (Chapter 4; Keogh 1983; Musgrave 1984). The most important effect for farmers is that animals gain weight more slowly, or even lose weight, and cannot be readily moved to non-toxic pastures. This places serious limitations on the management alternatives available to farmers (Everest 1983). It is these management practices (as outlined by Keogh 1983) which are primarily utilised to circumvent the ryegrass staggers problem at present. Direct monetary losses to farmers obviously vary considerably, but as an example, one Wairarapa farmer is estimated to lose between $60,000 and $80,000 annually, with up to 80% of a sheep flock affected (Broad 1986).

The complexity associated with *A. lolii* and its host-plant are further complicated according to the recent investigations by Barker (1987). He found the level of resistance afforded by *A. lolii*-infected ryegrass tissue was significantly reduced in the presence of infection by the vesicular arbuscular mycorrhizal fungus *Glomus fasciculatum*. In the absence of *A. lolii* the mycorrhiza had no influence on weevil feeding or oviposition, suggesting that the effect is an interactive one between the foliar and root endophytes, and not merely an effect of root infection by mycorrhiza. In field situations the role of mycorrhiza in the expression of ASW resistance has yet to be determined. Pasture grasses are invariably infected with mycorrhiza but there is considerable variation between sites in the mycorrhizal species and their incidence in roots (Mosse and Bowden 1968; Powell 1977).

This interaction between foliar and root endophytes requires further study at the biological and chemical level before the full relevance to ASW can be determined.

With respect to ASW control, the presence of endophyte is clearly detrimental to the insect, and so the perennials containing endophyte rather than endophyte-free perennials or annuals should be utilised in any managerial programme. The ryegrass cultivars which generally have a high level of endophyte in their seeds include Ellett, Yatsn 1, Droughtmaster, and Grasslands Nui. Only these perennial ryegrass cultivars should be used in permanent or long-term pastures, and the seed must be fresh as discussed above. These grasses should be established in autumn (March onwards) to develop before ASW attack. Most cultivars of Italian (annual) and short-rotation ryegrasses currently on sale (Manawa, Paroa, Tama and Moata) are highly susceptible to ASW because of the lack or inaccessibility of the endophyte to the weevils. These should be avoided unless there is a specific requirement for feed in the winter following sowing. It is because of this high winter and spring production of these cultivars, however, that they have a valuable role as winter sown, special purpose pasture, but they show very poor long-term persistence because of ASW attack. Two relatively new annual ryegrass cultivars, Concord and Progrow, contain strains of endophyte which do not protect the plants from ASW larval attack in spring and summer, although Progrow is resistant at the seedling stage to adult ASW attack in autumn. These cultivars should be used if winter feed is required. Mixtures of short rotation and perennial ryegrasses should never be sown in permanent pastures, and similarly, short rotation grasses should not be undersown into permanent pastures. Such mixtures often give high levels of winter and spring production initially, but the short rotation component of the sward allows ASW numbers to build up to high numbers, causing larvae to transfer to the companion perennial ryegrass plants when the short-rotation component is severely damaged and depleted. Even if these perennials have high levels of endophyte, larval feeding may still cause substantial damage in summer and autumn, necessitating resowing. In contrast, if the only ryegrass sown is a perennial cultivar with a high level of *A. lolii* endophyte, deterrence of the adult weevils prevents large numbers of eggs from being laid and populations remain at low levels. The plants therefore are never subjected to severe attack by larvae (East *et al.* 1987).

The desirability of sowing ryegrasses infected with endophyte or not must ultimately rest with the individual farmer who faces the predicament of whether to use endophyte-free perennial ryegrasses and accept loss of production and persistency due to ASW damage, or use endophyte-infected grasses and accept stock losses and inconvenience caused by ryegrass staggers.

As previously mentioned, it is doubtful if ASW can detect the presence of endophyte via olfaction unless the olfactory cues examined are masked by other factors such as humidity gradients (Chapter 3). A touch/taste system is more likely to operate. Feeding weevils (and those excavating oviposition tunnels) directly contact endophyte and/or endophyte related chemical compounds. This is presumably why oviposition responses parallel feeding responses (Chapter 4 and 5). Furthermore, the present study has demonstrated in bioassays with cut leaves and leaf extracts that *Acremonium*-infected ryegrass contains a feeding deterrent active against adult ASW. Partial isolation of the active fraction has confirmed the biochemical nature of the deterrent when compared with the results obtained by Gaynor and Rowan (1985), Rowan and Gaynor (1986) and Prestidge *et al.* (1985d). Gaynor and Rowan (1985), Rowan and Gaynor (1986), and Rowan *et al.* (1986) have isolated and named the deterrent "peramine", an alkaloid produced by the fungus which deters adult ASW feeding at 1 ppm, a concentration comparable to that found in *A. lolii*-infected ryegrasses (Rowan and Gaynor 1986). The molecular formula of peramine is $C_{12}H_{17}N_5O$ (Rowan pers. comm. 1986). Peramine is a basic hydrophilic, polar compound which is dialysable and therefore of low molecular weight. In contrast, the chemicals implicated in the ryegrass staggers disorder have been identified as tremorgenic neurotoxins, and named lolitrems (A, B, and C) (Gallagher *et al.* 1981, 1984, and 1985). These lolitrems are related to the known tremorgenic mycotoxins; alfatrems, penitrems, and janthritrems, in terms of structure, biogenesis, and biological effects (Gallagher *et al.* 1984; Siegal *et al.* 1985). The principle lolitrem associated with ryegrass staggers (lolitrem B) has the formula $C_{42}H_{55}NO_8$ (Gallagher *et al.* 1984). It is a highly lipophilic, non polar, neutral compound which contains a highly substituted indole ring system, and is thus very different from peramine.

As the lolitrems are not insect feeding deterrents (Prestidge and Gallagher 1985), insect resistance and animal toxicity in endophyte-infected ryegrasses could arise by different and potentially separable mechanisms, suggesting that by manipulating the biosynthetic processes in either the fungus or the plant, it may be possible to produce a ryegrass resistant to ASW which does not cause ryegrass staggers in livestock. On a similar line. Prestidge *et al.* (1985a and b), proposed that it might be possible to select perennial ryegrasses with sufficiently low levels of endophyte infection to maintain stem weevil resistance, yet reduce the ryegrass staggers producing component. Ryegrass staggers occurs when more than 25 mean counts of *A. lolii* hyphae/mm of leaf sheath width, whereas weevil activity is adversely affected by mean counts above 5 - 10 hyphae/mm (Prestidge *et al.* 1985b). Research is continuing along these lines at Ruakura (Prestidge pers. comm. 1986). Tying in with this is the identification of different strains of *A. lolii* (Latch pers. comm. 1987). It is likely that these strains differ in their ability to produce both peramine and lolitrems. Screening these strains for low levels of lolitrems but sufficient peramine levels to deter ASW is also under investigation (Latch pers. comm. 1987).

The utilisation of any form of genetically engineered ryegrass cultivar of superior resistance to ASW will unfortunately require considerable time before incorporation into the pastoral ecosystem. Present forms of control recognise the benefits of endophyte infections, although dilution of endophyte-infected ryegrasses with other grasses or legume species may be a useful way of reducing the severity and duration of ryegrass staggers (Keogh 1986). A number of new grass cultivars have been bred to complement and outproduce ryegrass at critical feed times (late summer - early autumn). Cocksfoot (*Dactylis glomerata*) is the most widely sown grass after ryegrass (Sangakkara *et al.* 1982), and is usually sown with ryegrass. Once established it can compete well with ryegrass and generally persists well under rotational grazing (Lancashire and Brock 1983). Tall fescue (*Festuca arundinacea*) is also a most useful grass on peat soils where it shows superior summer production to ryegrass. However, both are particularly susceptible to ASW attack. Furthermore, sowing endophyte-infected ryegrasses with other susceptible grasses may only enhance the ASW population buildup leading to a depletion of the ryegrass. Larval feeding activity is not affected by the presence of *A. lolii* in the diet (Prestidge *et al.* 1985b). Larvae could thus transfer from the susceptible grasses to feed on endophyte-infected ryegrasses. In fact, the presence of larval toxins and growth retardents in endophyte-infected ryegrass (e.g. lolitrem B (Prestidge and Gallagher 1985)) may actually exacerbate plant damage as larvae would require more endophyte-infected tillers to complete development (Prestidge *et al.* 1986). This reinforces the importance of adult deterrency as the primary source of endophyte related resistance. Any pasture grass mix may therefore lead to a weaker and less productive pasture after stem weevil attack than if endophyte-infected ryegrass/white clover pasture was initially sown. In these situations insecticidal applications are necessary, especially over summer in the first year of pasture establishment. This is because grasses such as cocksfoot, Phalaris (*Phalaris aquatica*), timothy (*Phleum pratense*) and browntop (*Agrostis tenuis*) are susceptible to ASW (Pottinger 1961a; Barker *et al.* 1984a) due to oviposition and transfer of larvae from weed grasses such as *Poa annua*, which infests badly managed pastures or pastures damaged by pests such as grass grub. Successful farming without reliance on permanent *L. perenne* based pastures is possible in arable areas, but in other parts of New Zealand emphasis on all grass farming demands persistency of pasture species in order to sustain high and acceptable levels of pasture production, and hence *L. perenne* based pasture is essential.

Further long term opportunities exist to genetically engineer *Acremonium* spp. genes into other grasses lacking resistance to pests (Pottinger *et al.* 1985). Manual inoculation of endophytes into endophyte-free seedlings has already been accomplished (Latch and Christensen 1985). Genetic engineering could be enhanced by breeding for greater tolerance against ASW and other pests in grasses other than *L. perenne* included in pasture mixtures. Tolerance associated with endophyte would improve the control of ASW and ensure higher levels of pasture production, whilst increased tolerance in species and cultivars lacking endophyte would assist their persistency in mixed swards (Pottinger *et al.* 1985).

Perhaps with this multidisciplinary approach by plant pathologists, biochemists, entomologists, plant geneticists and breeders, the dilemma to the ASW/ryegrass staggers problem facing farmers with respect to which pasture grass to grow may eventually be simplified.

The identification of endophyte goes a long way to explaining much of the earlier contradictory evidence on the susceptibility to ASW of various pasture grasses. However, Gaynor and Hunt (1982 and 1983) and Hunt and Gaynor (1982) defined two factors apart from endophyte which could affect the degree to which a ryegrass cultivar may be damaged. Firstly, environmental factors such as moisture stress and soil fertility (as discussed in Chapter Two), and secondly, the genotypic differences between cultivars. With equally low endophyte levels, short-rotation ryegrasses with a *L. multiflorum* X *perenne* genotype such as "Manawa", were more susceptible than perennial ryegrasses with a *L. perenne* genotype. Similar effects were evident in the present study, in both feeding (Fig. 1) and oviposition (Fig. 9) responses of ASW to various ryegrass cultivars. These differences could thus reflect possible genotypic variations between the ryegrass cultivars. Gaynor and Hunt did not attempt to elucidate these factors, so several were investigated here. With respect to feeding these were leaf strength and leaf fibre content, and with respect to oviposition were age of the tiller, tiller hardness, fibre content of the tiller region, and tiller diameter. Results from the leaf lamina strength trials indicated that the annual ryegrass cultivars were weakest, registering tensile strengths for the 5 cm section of leaf tested of less than 150 g/mg. The perennials were stronger, with tensile strengths between 150 and 200 g/mg. Further substantiating evidence was obtained from the neutral-detergent fibre analysis, as fibre is correlated to leaf strength (Evans 1964) which imparts toughness to ryegrass leaves (Langer 1973). Most fibre was contained within perennial leaf lamina tissue, with the least amounts in annuals. The preference of feeding weevils for the annual cultivars over perennial cultivars (without endophyte) can thus be partially explained through the leaf fibre content and consequent strength. Unfortunately, feeding weevils tend to feed selectively around the fibrous tissue of leaves, so any form of genetic manipulation to breed tougher ryegrasses which deter feeding weevils would likely be ineffective. It would also be impractical, as the tougher plants are also less palatable to grazing livestock (Evans 1964).

The ovipositing female probably represents the most important phase in the host-plant selection process for ASW. Any form of deterrence that prevents egg laying is thus very important. As already discussed, endophyte mycelium, being concentrated within the sheath region, and/or associated chemicals, are good deterrents. However, genotypic factors may also be important. Fibre content within the sheath region was examined, which again showed that the perennial cultivars contain more fibre than the annuals, which parallels ASW's oviposition preferences. This could result in ASW experiencing greater difficulty excavating oviposition holes, although this could not be substantiated when testing for tiller hardness with a penetrometer. However, the same argument against breeding tougher leaf material discussed above also holds for breeding tougher sheath tissue.

Tiller diameter was another genotypic factor which showed a consistent relationship to oviposition preference. The larger diameter tillers were preferred oviposition sites over small diameter tillers, which correlated with preference for perennial and annual ryegrass cultivars. Substantiating evidence for the importance of tiller diameter was provided by the responses of weevils to various diameter glass tubing. Weevils preferred to climb the larger diameter over the smaller diameter tubing. Whether this preference is a result of differences in leaf vernation between the perennials and annuals, or simply due to the increase in surface area a cheap form of control in the long term, if the corresponding agronomic characteristics are suitable for livestock. Conversely, breeding larger diameter perennial ryegrass tillers may provide improved oviposition sites for ASW, which, if combined with endophyte, may severely decrease ASW predation. To be of increased value, these non-preference forms of resistance should be amalgamated with antibiotic forms of resistance, so an insect "choosing" a specific host-plant will also suffer adverse antibiotic effects.

The possibility also exists for the discovery of regional ecotypes of ryegrasses which are of superior resistance to ASW. In the past, such cultivars as Nui and Ellett were both naturally occurring ecotypes from Mangere, south Auckland (Armstrong 1977; Duder 1986). Similarly, Ruanui is the result of blending ecotypes from the Hawkes Bay / Poverty Bay regions (Corkill *et al.* 1981). Two recently released cultivars were also localised ecotypes, Droughtmaster (previously Takapau, Kain *et al.* 1982a and b), from Hawkes Bay, and Concord, from Waikato. More regional ecotypes may thus exist which exhibit superior resistance to ASW. Where identified, the nature of such resistance should be investigated.

"As greater understanding of insect and plant biology, chemistry, and ecology is attained, we will be able to approach the goal of developing economic plants that are deliberately and foresightedly designed to be insect resistant", Beck (1965). This thesis provides fundamental information on the behavioural responses of ASW and possible plant factors responsible for such behaviour. It is evident that ryegrass infection by *A. lolii* offers partial but not absolute resistance to ASW. Some feeding and oviposition will occur on *A. lolii*-infected plants, even in the presence of non-infected hosts. Factors such as host-plant genotype (Chapters 4 & 5), variations in hyphal content of individual plants (Prestidge *et al.* 1985b), different strains of *A. lolii* (Latch pers. comm. 1987), as well as infection by other endophytes and preferences amongst individual weevils (Gaynor *et al.* 1983; Barker 1987) may influence the level of resistance. Furthermore, sward management and soil type factors influence the interaction between *A. lolii* incidence and ASW damage in the field (Barker *et al.* 1986). Implementation of compatible biological and cultural control methods in association with insecticides would ultimately reduce the impact ASW has upon the N.Z. pastoral ecosystem, which in turn could lead to both increased herbage production and increased animal production.

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