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THE EPIDEMIOLOGY OF A NEW LEATHERJACKET PEST (*Tipula oleracea*) OF WINTER CEREALS IN NORTHERN BRITAIN

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THE EPIDEMIOLOGY OF A NEW LEATHERJACKET PEST *(TIPULA OLERACEA)* OF WINTER CEREALS IN NORTHERN BRITAIN

by

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SUMMARY

Leatherjackets are the larvae of craneflies, or daddy long-legs, and are known pests of spring sown cereals in northern Britain. The factors causing damaging populations are well established. The timing of the crop is such that seedlings coincide with the later stages of larval growth when consumption per leatherjacket is at its maximum and so relatively few leatherjackets (>280,000 ha^{-1}) can cause yield loss that is worth controlling with an insecticide. The biology and behaviour of the species involved, *Tipula paludosa*, favours existence in a stable habitat such as grassland which allows numbers to increase over a few seasons. Thus, generally, only those spring sown crops which follow grass are likely to have leatherjacket populations above the economic threshold.

In the mid 1980s a number of farmers reported autumn damage by leatherjackets in winter cereal crops, sometimes leading to crop destruction. There were a number of features of these attacks that suggested that *T. paludosa* was not responsible. Prominant amongst these was the observation that relatively large larvae (third and fourth instars) were to be found in November; at this time of year *T. paludosa* larvae are in their second instar. Additionally, very large populations (> 2m ha^{-1}) were recorded and these were unexpected in cereals, even though such numbers are sometimes seen with *T. paludosa* in grassland. There was thus reason to believe that the attacks on winter cereals were not attributable to *T. paludosa*.

A survey of crops with reported autumn leatherjacket damage revealed that 84% had been preceeded by a crop of winter oilseed rape. This argued strongly that an explanation for this new pest problem should be sought in a study of the interactions of leatherjackets with rape rather than an investigation of their effect on winter cereals. Such a study was complicated by the inability to consistently distinguish between leatherjackets of the closely related species *T. paludosa, T. oleracea* and *T. subcunctans*. A means of reliably identifying larvae was a precursor to understanding the problem.

Thus the project set a number of objectives:

To differentiate between agriculturally important leatherjackets at all stages of development.

To develop easy and reliable sampling methods for leatherjackets within cereal crops and stubbles.

To investigate records of the occurrence of leatherjackets and leatherjacket damage in winter cereals and identify predisposing factors.

To investigate the influence of oilseed rape upon leatherjacket population dynamics.

To elucidate the annual cycle of the new pest species.

A study of protein bands from eggs, larvae, pupae and adults of *T. paludosa* and *T. oleracea* using isoelectric focussing showed that there was a consistent difference between the two species. This held true for all stages, irrespective of whether specimens contained food or were parasitised, and the technique worked for specimens collected from regions of Europe. Under this system, *T. subcunctans* could not be separated from *T. paludosa* but this was not important since this species is rare and overwinters as eggs; the lifecycle is sufficiently different that it cannot be implicated in damage to either winter or spring cereals.

Application of the isoelectric focussing method to leatherjackets recovered from winter cereal crops proved that they were *T. oleracea* and confirmed that *T. paludosa* was not responsible for the damage.

It was thought that it may be necessary to be able to identlfy leatherjackets in the field. Attempts to use the difference in proteins between the two species to develop an antibody-based system for this purpose were, however, unsuccessful.

A series of 15 experiments was made to compare different sampling methods for use in arable crops. The most effective and easiest to use was brine flotation. This technique involves the insertion of 10 cm diameter plastic pipes into soil and the application of brine solution. Any leatherjackets encompassed by the pipes float to the top where they can be counted. Brine flotation is unreliable on cultivated soils but is effective later in the season when soils are more compact.

Leatherjacket distribution studies were carried out in Scotland and Northern Ireland. They showed that whilst *T. paludosa* was easily found in agricultural land, *T. oleracea* is only rarely encountered as larvae. It was not possible to discover the larval habitat of *T. oleracea*. The occurrence of this species in dense populations, as seen in winter cereals, is therefore unusual.

Samples collected from winter oilseed rape and nearby fields of winter wheat yielded *T. oleracea* larvae in 20% of the rape fields but none in the wheat during November. In the spring, leatherjackets were found in the wheat but more rape fields contained them and by September they were detectable in 73% of the rape fields. Numbers of leatherjackets recovered over this sampling period increased in the rape but not in the wheat.

Laboratory studies showed that *T. oleracea* larvae could survive on a diet of oilseed rape and would grow faster on rape than if fed wheat.

By the time of the first flight period of the year for *T. oleracea* (May/June), the rape canopy has closed over. Field experiments showed that the canopy impeded the vertical movement of adult craneflies but not horizontal movement. The rape crop therefore prevented the normal dispersal behaviour of *T. oleracea*.

These results provide an explanation for the new pest problem of leatherjacket damage to winter cereals. The female *T. oleracea* is a relatively strong flier and chooses to lay eggs in seedling rape crops in the autumn. The eggs hatch and the larvae are able to thrive within the crop. By the time they are full grown and pupate, the rape canopy is sufficiently developed to prevent emerging adults escaping from the crop; they are therefore obliged to mate and lay their eggs beneath the canopy. Thus a second generation occurs within the rape crop and these are the leatherjackets that are present, often in large numbers, when the following winter cereal is drilled.

This new leatherjacket pest has arisen because oilseed rape has been introduced into arable rotations and this is the key to understanding the problem. By the time that leatherjacket damage is seen in the winter cereal, it may be too late to effect control. Farmers are therefore recommended to sample rape stubbles before cultivations begin. The brine flotation method provides an easy to use and robust sampling technique with which to estimate leatherjacket numbers. Adopting this approach anticipates leatherjacket attack and maximises the time available to apply control measures.

1. INTRODUCTION

Craneflies, colloquially known as Jenny or Daddy long-legs, are easily recognised as large flies with slender bodies and long legs. Craneflies can be readily distinguished from several other similar families by the absence of ocelli, the presence of two anal veins and a vein shaped suture dividing the praescutum from the scutum (Coe *et al.*, 1950). Craneflies belong to the family Tipulidae, sub family Tipulinae, Genus *Tipula* - the long palped craneflies. The Tipulidae is the largest Dipteran family in the UK and contains 303 species (Smith, 1989). The larvae of the group can be found in a wide range of habitats including decaying wood, moss, marsh land, mountainous areas and agricultural land (Brindle, 1957). The common feature of the larvae is that they are found in moist environments; the larvae of some species are wholly aquatic with one species, *Geranomyia unicolor,* breeding in salt water (Coe *et al,* 1950).

Despite this large number of species, only a handful are reputed to cause damage to agricultural and horticultural crops. *Nephrotoma flaxescens, N. appendiculata* (Jones and Jones, 1984), *Tipula variipennis*, *T. vernalis* and *T. subnodicornis* (Blackshaw, 1992) are occasionally cited as minor pests but the two species most frequently referred to are *Tipula paludosa*, the marsh cranefly and *Tipula oleracea,* the cabbage cranefly. The larvae of these two species are often considered together under the descriptive term, leatherjackets. Authors of past papers have recognised *T. paludosa* as the more important of the two in terms of agricultural impact (Blackshaw, 1985; Brownbridge and Selman, 1989) but few have actually distinguished between the species in the field; most authors refer to leatherjackets as the larvae of *Tipula* species (White and French, 1968; Tucker and Cutler, 1975; Blackshaw and Newbold, 1987).

Adult craneflies present no concern in terms of crop damage as they do not feed on plant material. The larvae, on the other hand, are recognised by farmers, advisors and gardeners as a serious threat to plant establishment (Anon, 1984). They are considered a major pest of permanent grassland, newly reseeded grassland and spring cereals (French, 1969; Rayner, 1969; Newbold, 1981). The larvae spend the majority of the winter months in a relatively quiescent state and feed voraciously at the onset of spring temperatures. Spring cereals suffer on an annual basis; the larvae feed on emerging shoots, cutting them just at, or below, ground level. The presence of a ragged edge at the point where the plant has been severed is frequently indicative of leatherjacket feeding. Occasionally leatherjackets will feed on the leaves and they can also hollow out the grain (White and French, 1968) which subsequently prevents germination. This results in the appearance of bare patches in the field, which is often the first sign of damage that is noticed by the grower.

Grassland suffers most heavily from leatherjacket damage. Grass reseeds show similar symptoms to spring cereals, young shoots are snipped off at the emergence point and as a result bare patches are obvious. Damage symptoms are much more apparent on young establishing plants which can make no attempt to compensate for damage. By comparison, damage is not always so obvious on well tillered, permanent, pasture however established swards can suffer when the larval population is high and damage can range from complete sward destruction (White and French, 1968) to insidious levels of damage which are hardly detectable (Blackshaw, 1984).

Blackshaw (1985) estimated that leatherjackets were responsible for in excess of fifteen million pounds worth of damage in grassland in Northern Ireland annually and White and French (1968) recorded yield increases of 74 percent following control of populations of 4.0-4.5 million larvae per hectare. However Table 1.1 shows that leatherjackets are not restricted to cereal crops and grassland and the reports of leatherjacket damage from a variety of crops confirms that they are omnivorous in their feeding habits.

Table 1.1 Summary of crops attacked by leatherjackets

Crop attacked	**Author**
Grass	Blackshaw, 1984, 1985
Spring cereals	Golightly, 1967, French, 1961, 1966 Rayner, 1969, Newbold, 1981
Swedes	Anon, 1981
Potatoes	Anon, 1989
Turnips	Cameron, 1917
Strawberries	Theobald, 1929, Alford, 1984
Loganberries, blackberries, raspberries	Alford, 1984
Maize	Gair *et al.*, 1983
Clover	Mowat, Shakeel, 1988
Brassica crops, peas, beans, lucerne, carrots, celery, lettuce	Anon, 1953
Sugar beet, sweet-corn	Mc Kinlay, 1992
Berries, tobacco, various vegetables and ornamentals	Campbell, 1975

Although previous researchers have acknowledged that two species of leatherjackets could be found in grassland, reports of leatherjacket damage in the

literature rarely distinguish between the larvae responsible and this is largely due to the difficulty in obtaining an accurate identification. Part of the problem in distinguishing between the species is that the larvae are morphologically very similar (Theowald, 1957, 1967; Brindle, 1959). They are elongated, cylindrical, a little tapered anteriorly and truncate at the rear (Coyler and Hammond, 1951). The body is enclosed in a tough integument which gives rise to the name by which they are most commonly known 'leatherjackets'. There are four instar stages which vary in weight and size and these differences further add to the complexity of separating the larval stages. Brindle (1959) produced notes on the differentiation of the larvae and in 1960 presented a key to determine between the species. The differentiation between the larvae of *T. oleracea* and *T. paludosa* centres round examination of the anal papillae as well as noting slight differences in size and colour. In practice these keys have not met with consistent success when used by researchers in the field (R.M. Stewart; R.P. Blackshaw, unpublished observations). As a result the life-cycle and habits of field populations are almost always accorded to *T. paludosa,* which is by far the most common species found on the wing.

At the beginning of the twentieth century there was also confusion over the identification of the adults of the *T. oleracea* group. Theobald (1913) and Rennie (1916) believed that *T. paludosa* had two generations per year in England. However it is likely that reports of the earlier generation were actually *T. oleracea.* The controversy over the identification was finally settled by De Jong (1922; 1925) who successfully separated the adults of each species and added *T. czizeki* (now *T. subcunctans*) to the group.

By comparison to the larvae, the adult stages of *T. paludosa, T. oleracea* and *T. czizeki,* although quite similar in appearance at first inspection (Den Hollander, 1975), can nevertheless be reliably separated by several physical attributes. The distance between the eyes, the number of antennal segments and the wing length can be reliably used to discern between the adults (Coe *et al.,* 1950). The wing length to body ratios are so distinct that it is reasonably easy to identify between female *T. oleracea* and *T. paludosa* in the field.

Researchers, when referring to the life cycle of leatherjackets, are generally alluding to the life-cycle of *T. paludosa* which has been well documented (Rennie, 1916; 1917; Barnes, 1936, Laughlin, 1967). Previously there may have been no real need to separate the two species as, for the most part, leatherjacket damage has consistently followed a predictable pattern which has fitted in with the known life cycle of *T. paludosa.*

Tipula paludosa has one generation per year. The adults emerge from pupation from June to September (Rennie, 1917). Female adults are gravid at emergence and

are not able to fly far (Dobson, 1974). Mating occurs soon after emergence and 75% of eggs are laid before daybreak (Coulson, 1962) in one batch amongst herbage, particularly grasses. Before the eggs are laid the female is unable to fly any distance (Rennie, 1916). Dispersal is therefore limited and local population concentrations are common. The larvae hatch out after about 14 days and are a pale sandy colour. The body consists of thirteen segments; the spiracles are found on the last segment and in the first instar small tufts of bristles can also be seen here. The larvae start to feed on the bases of stems and roots immediately. The winter is spent in the second or third instar stage. They can be active at temperatures as low as 5°C (R.P. Blackshaw, unpublished data in Blackshaw, 1992) and so can continue to feed during periods of mild weather. They become more active and feed voraciously in the spring when the soil begins to warm (Laughlin, 1967). When fully grown, they reach approximately 3-4 cm in length and they stay within their burrows for about 6-8 weeks before pupating. They remain as pupae for approximately two weeks. In total the larvae spend a minimum of nine months in the soil (Rennie, 1917).

By contrast the information collated on the life cycle of *T. oleracea* is not so definitive and indeed some of the past observations of leatherjackets in 'unusual' situations (Anon, 1952) might in fact have been attributed to differences in the life cycle of *T. oleracea* compared with *T. paludosa.* Despite its recognition as an agricultural pest, no records can be found in the literature where *T. oleracea* has been recorded independently from *T. paludosa.*

Generally speaking *T. oleracea* is not a particularly abundant species and it is certainly less common than *T. paludosa* (Coe *et al.,* 1950). Where researchers have trapped adult flies using either light traps (Pinchin and Anderson, 1936; Dobson, 1974) or water traps (Blackshaw, 1983) the total numbers of adults caught are always low in comparison to *T. paludosa.* It has been observed that female *T. oleracea* is a stronger flier than *T. paludosa* (Coulson, 1959) and is able to fly when gravid. The female lays her eggs in several batches (Cuthbertson, 1929) and this, combined with their ability to fly well, implies that they lay their eggs over a wider area than *T. paludosa.*

The flight period recorded for this species, both in the UK. and abroad, begins to shed further light on the difference between the two species. Like several other European tipulids, namely *T. meditterranea*, *T. orientalis* and *T. plumbea* (Den Hollander, 1975), *T. oleracea* has frequently been reported as a bivoltine species with a spring generation and a late summer/autumn generation. In Italy, Del Guercio (1914) recorded adults flying in mid- March and again in September to October, in Russia, Silant' Ev (1931) reported flights from July to October; Sellke (1936) noted flights in Germany from May-June and again in September/October. In the UK, Miles (1921) observed two distinct flight periods at the end of April through to June and

again from September to October. The variation in flight periods is probably due to the difference in climatic conditions and it is thought that the number of generations may vary between years and within localities (Den Hollander, 1975). This variation may be sufficiently great to result in only one marked flight period in any year.

Rennie recorded *T. oleracea* in Aberdeenshire in 1917 but only in small numbers by comparison to *T. paludosa.* Milne (1966) caught adults easily in Northumberland using a light trap but admits to little success in ascertaining the larval habitat despite repeated searches. Despite this inability to pinpoint the whereabouts of *T. oleracea* larvae the general consensus of opinion would appear to be that the larvae were present in grassland. Again this may however, be related in some way to the fact that researchers working with field populations have been unable to differentiate between the larvae but were able to trap adults of both species in traps set near grassland sites. The difficulty in dealing with the information pertaining to leatherjackets is that there is often no way of confirming which species is actually the subject of some of the literature and therefore it seems appropriate to consider the available information on leatherjackets, whilst high-lighting that which is particular to *T. oleracea.*

The fact that *T. oleracea* has been shown to have a bivoltine life cycle immediately suggests that the larvae do not spend as much time in the soil as *T. paludosa* and that therefore the life cycle described for leatherjackets is not appropriate for *T. oleracea.* Furthermore the literature shows that much of the laboratory work carried out on leatherjackets has been carried out on *T. oleracea* (Carter, 1972; Carter, 1973; Vlug, 1990). This is because the life cycle can be more easily manipulated than that of *T. paludosa.* Laughlin (1960) showed that *T. oleracea* could complete the whole life-cycle from egg to adult stage in an average of 11-12 weeks if kept at a constant temperature of 21°C. By contrast *T. paludosa* have a reputation for being difficult to culture in the laboratory; unlike *T. oleracea* they have a diapause period and because they spend a longer period of time in the soil they are likely to incur larger mortalities

In an attempt to evaluate potential targets for biological control Stich (1962) studied the courtship pattern of *T. oleracea* in some detail. Courtship appears to involve a series of stimulus response reactions, namely, grabbing, mounting, pinning, searching and kissing followed by a sliding reaction. This routine appears to be fixed for this species and copulation will not take place if the procedure is not carried out in this exact sequence. Only active males and unfertilised, receptive females are able to complete the whole process, subsequently this prevents mating between males, fertilised females and other species. Furthermore, Pinchin and Anderson (1936) showed that the nocturnal activity of *T. oleracea* was different from that of *T. paludosa* but that female and male activity of each species did coincide; presumably

this is also an important feature of the mating process. In addition to this, Downes (1969) found that female *T. oleracea* was attracted to the male by hormones.

Due to the impact of leatherjackets as agricultural pests, factors affecting survival have been well studied. The climate and especially soil moisture plays an important part in the survival of the larvae. The eggs and the larvae of both species are sensitive to desiccation, the eggs of *T. oleracea* particularly so (Meats, 1972). Milne *et al.* (1965) concluded that a shortage of moisture in September and October, at egg laying time, resulted in a low larval population that autumn and French (1969) also suggested a relationship between larval survival and rainfall. Similar conclusions were reached by White (1963). However Blackshaw (1983) found that autumn larval populations had a negative relationship with summer and autumn rainfall in Northern Ireland and suggested that there are a number of positive and negative factors which act to sustain and suppress the population.

Certainly there seems to be a delicate balance between the survival of the larvae of both species and the moisture content of the soil. Despite being susceptible to desiccation and being more readily found in heavier soils, mortality has been induced by soil flooding (Meats, 1970) to such an extent that soil flooding is a recommended control procedure in some parts of the world (Anon, 1986). Meats (1970) showed that larvae will survive in pure water until they starve and suggests that some reaction between the soil and water is responsible for the death of the larvae. Deoxygenation may play a part but other factors such as nitrites, ammonia, nitrogen gas and a variety of toxic sulphides are thought to have some role, although this has not been evaluated.

The population of leatherjackets in some areas has been shown to fluctuate from year to year with peak years being observed at approximately 5 year intervals (Cohen, 1953; White, 1963) and it is thought that this is linked to environmental factors. The characteristics of each individual pasture also appear to influence the size of the leatherjacket population. McCracken *et al* (1995) found that the leatherjacket population in pasture land in the west of Scotland could be related to three environmental variables - aspect, distance from the Atlantic and tendency to waterlogging - three farm management variables, silage use, use of organic fertiliser and sward height during winter, -and the number of leatherjackets in the pasture in the previous year. It was thought that these variables affected the leatherjacket population through their association with the soil moisture and microclimate and sward productivity and nutrient content.

Although the climate undoubtedly plays an important feature in the regulation of the population there are many natural agents which can exert some pressure on the larval population including other insects, vertebrates, viruses, bacteria, nematodes and fungi

(Table 1.2). However, whilst these factors may have some influence they are not considered to be of major importance in the regulation of the population.

Cannibalism has been observed among the larvae (Barnes, 1936; Laughlin, 1958; Freeman, 1966; Ahmed, 1968). The larvae are armed with large mandibles and these have been described not only as tools for feeding but also as weapons of offence. In laboratory experiments fatal attacks upon each other were recorded at three percent per day (Freeman, 1966). The larvae will not only attack and feed on other healthy larvae but they will also feed on the cadavers if they encounter them and this is an important behavioural feature in the transmission of *Tipula* iridescent virus (TIV) (Carter, 1973). The effect and the reason for cannibalism has been disputed; Bodenheimer (1923) suggested that it was a reaction to feeding deficiency, whilst Sellke (1936) argued that this was not the case and that cannibalism was the exception for some larvae and not the rule and that furthermore it was probably density dependent.

Given the list of predators which can feed on the larvae of *Tipula* spp., it must also be recognised that these larvae play an important role in the food chain of other animals; including mammals such as shrews, hedgehogs and moles. Not all scientists are in agreement with the need to reduce the population of these species and research suggests that leatherjackets may be a vital component in the diet of birds (Bentena *et al.,* 1991, McCracken *et al.,* 1992). In some areas there may be a move to encourage leatherjacket populations to prevent the demise of species, such as the chough in Islay (McCracken, 1990). There is also some concern that the insecticidal control methods employed are not specific and may have deleterious effects on other soil invertebrates and predators (Aveling, 1977; 1981; Jepson *et al.,* 1975; Vickerman, 1988). In response to these concerns Clements *et al.* (1988; 1992) showed that the effect of chlorpyrifos on birds and mammals was minimal both where the birds were feeding directly on treated grass and also when the birds fed on affected invertebrates.

As the damage caused by leatherjacket feeding can result in economic loss researchers have developed treatment thresholds as an advisory tool. These thresholds vary between and within crops depending on the physiological age and the nutritional level of the plants. As a general rule, populations of over 1 million ha^{-1} in permanent grass and 600,000 ha^{-1} in spring cereals would normally warrant an insecticide treatment (Anon 1994). Leatherjacket populations have been monitored in the UK by ADAS, DANI and SAC, using a variety of soil sampling techniques. SAC uses the Blasdale technique to monitor soil populations of leatherjackets. Soil samples are taken using a corer and the samples are returned to the laboratory where the larvae are extracted by heat (Blasdale, 1974). When the larvae have been collected a population estimate can be made. Once these figures have been collated they may serve as a

warning of high risk years or they may highlight particularly susceptible localised patches. Alternatively a DIY kit, developed by Stewart and Kozicki (1987) is sometimes used by farmers and advisers. Neither system differentiates between the species.

An alternative system of forecasting has been developed by Blackshaw (1990) and Blackshaw and Perry (1994). This system relies on a climate based multiple regression model which allows an estimation of the mean annual population and the frequency distribution of field populations, to be made when the annual data are fitted to an Adès distribution. However although this method has proved to be effective at estimating the annual mean population and the frequency distribution of field populations, it has not yet been developed to forecast the population in individual fields and this is important in terms of reducing insecticide use and costs to individual growers. Similarly the attempt by McCracken *et al* (1995) to model leatherjacket numbers in individual fields was unsuccessful but it may provide some useful pointers for the future.

Leatherjacket control relies quite heavily on insecticide applications but some cultural methods can reduce damage. Initial control methods on golf courses relied on the fact that leatherjackets respond to excessive moisture in the soil by coming to the surface; greens were deliberately soaked and the larvae were trapped under tarpaulins (Morison, 1951). In agricultural systems it has been demonstrated that although attacks are frequent on crops which follow grass in the rotation these attacks can be prevented if the grass is ploughed in July or early August and the herbage is well buried (Anon, 1984). Applications of nitrogen are also thought to help the plant compensate for feeding damage (French, 1969). However these methods are seldom sufficient under heavy attacks and consequently control methods have favoured the use of a variety of insecticides in grassland and spring cereals.

DDT was widely used against leatherjackets but since this was banned in 1984, the only organo-chlorine insecticide currently approved for the control of leatherjackets is gamma-HCH and this insecticide is presently due for review by the Pesticides Safety Directorate. Organo-phosphate insecticides such as fenitrothion, quinalphos and triazophos are approved for use in grassland but chlorpyrifos is probably the chemical most favoured (Clements *et al.*, 1992) by growers and advisors. One of the carbamate insecticides, methiocarb, is claimed to reduce the leatherjacket population in cereals but does not provide effective control. The insecticide itself is not the only important feature of control; timing of the application has been shown to have significant effects on the larval population and yield responses (Blackshaw, 1984; Mowat and Jess, 1986; Newbold, 1981). More recently biological control methods such as *Bacillus*

Table 1.2. Natural enemies of Tipula species as reported in the literature

Phyla	Species/Family	Development stage affected	Author
Vertebrate	*Talpa europaea* *Sturnus vulgaris* *Ciconia* sp. *Corvus* sp. *Vanellus vanellus*	Larvae	Theobald, 1903 Sellke, 1936 Feare, 1984
	Sturnus vulgaris *Pyrrhocorax pyrrhocorax* Waders	Larvae	Kluyver, 1933 Dunnet, 1955; 1956 Dunnet & Paterson, 1968 Barbash *et al.*, 1991 Beintena *et al.*, 1991 Mc Cracken *et al.*, 1992
Insects	Tachinidae	Larvae	Carter, 1976 Maercks, 1953
	Siphona geniculata (de Geer)	Larvae	Rennie & Sutherland, 1921 Carter *et al.* 1981
	Phaonia signata (Meigen) (Dipt. Muscidae)	Larvae	Griffiths *et al.*, 1984
	Pterostichus melanarius	Larvae	Chapman, 1994
Nematodes	Mermithidae, Thelastomatidae	Larvae	Rennie, 1924 Carter, 1976
	Neoaplectana carpocapsae	Larvae	Lam & Webster, 1972
	Panagrolaimus tipulae n. sp *Rhabditis tipulae* n. sp.	Larvae	Lam & Webster, 1971
	Sterneima feltiae	Larvae	Peters & Ehlers ,1994
Fungi	Entomophtoraceae	Larvae Adults *T.paludosa*	Muller-Kogler, 1957
	Entomophtora arrhenoctona	Adults *T. paludosa*	Keller, 1977
	Entomophtora gigantea *Empusa caroliniana*	Adults	Keller, 1978
Protozoa	Gregarinidae *Diplcystis tipulae* n. sp	Larvae	Carter, 1976 Sherlock, 1979
	Coccidia Microsporidia	Larvae	Carter, 1976
	Rickettsiella tipulae n.sp	Larvae	Muller-Kogler, 1958
	Entamoeba	Larvae	Mackinnon, 1912
Virus	*Tipula iridescent virus* (TIV)	Larvae; *T. oleracea* *T. paludosa*	Ricou, 1975 Carter, 1976 Carter, 1978
	Tipula nuclear polyphydrosis virus (NPV)	Larvae	Carter, 1976
Bacteria	*Bacillus thuringiensis* var. *thuringiensis*	Larvae *T. paludosa*	Lam & Webster, 1972
	Bacillus thuringiensis var. *israelensis*	Larvae	Waalwijk *et al.*, 1992

thuringiensis (Waalwijket *et al.* 1992), predatory nematodes (Peters *et al.,* 1994) and neem oil extracts are being investigated as control methods for leatherjackets but these products have not reached the stage where they are effective, or economically viable, for use in the field.

Leatherjacket damage is normally seen in the spring, however in the late eighties leatherjackets were reported in a 'new situation' in the north and east of Scotland and in Northern Ireland. Farmers, advisors and consultants reported some unusual observations.

Leatherjacket larvae were found causing damage in October - November in crops of winter cereals. These larvae ranged in size from second to fourth instar; in some cases large populations, exceeding two million larvae ha^{-1}, were found; even small populations, less than 650,000 larvae ha^{-1}, seemed to cause significant economic damage. Growers were unable to achieve consistent success with the insecticides which were commonly used for leatherjacket control.

In 1989 the damage caused by these larvae was so significant that some fields of winter wheat were unlikely to produce an economically viable crop and as a result the crops had to be ploughed in. Other growers reported losses of 2-4 tons per hectare. Although leatherjackets are commonly recognised as an agricultural pest, their occurrence in this situation could not have been anticipated from the knowledge of the accepted life-cycle.

The timing of the damage, the size and numbers of the larvae and the cropping patterns involved did not concur with the characteristics of leatherjacket damage as they were portrayed in the literature. Given the fact these observations had been noted over several years it appeared that this was not a unique situation but rather a new development in the pest potential of leatherjackets. When the larvae, collected from these fields were reared through to the adult stage they were identified as *T. oleracea* and not *T. paludosa*, the cranefly which is the species most frequently held responsible for crop damage (Blackshaw, 1988; C. Coll, unpublished data). It appeared then that a 'new ' pest scenario had arisen.

It was noticed that in the majority of cases of leatherjacket damage in winter cereals reported to SAC and DANI the preceding crop was oilseed rape (*Brassica napus*), and it was postulated that the oilseed rape had some influence on these leatherjacket populations.

Given that the area of oilseed rape grown in Scotland has increased more than six hundred fold it (Anon, 1994b) was thought that if the advent of oilseed rape did influence the development of leatherjackets then this might at least explain why *T. oleracea* damage to winter cereals is a relatively recent phenomenon. It was not clear

what the connection between oilseed rape and *T. oleracea* could be and so this study was instigated to examine any possible factors which may influence the appearance, development, reproduction or growth of *T. oleracea* .

The information available clearly indicates that *T. oleracea* is not a new species, but it has become a threat to the establishment of winter cereals in Scotland and Ireland. It would appear that *T. oleracea,* like most pest species, may have adapted to a change in land use which in turn has allowed it to increase its pest status. The appearance of these larvae has coincided with the advent of oilseed rape as a major crop in Northern Britain. Given the limited knowledge of the insect involved there is a need for information on the epidemiology of *T. oleracea* and its interaction with oilseed rape before the risk from this pest can be evaluated.

2. OBJECTIVES

1) To differentiate between agriculturally important leatherjackets at all stages of development.

2) To develop easy and reliable sampling methods for leatherjackets within cereal crops and stubbles.

3) To investigate records of the occurrence of leatherjackets and leatherjacket damage in winter cereals and identify predisposing factors.

4) To investigate the influence of oilseed rape upon leatherjacket population dynamics.

5) To elucidate the annual cycle of the new pest species.

3. DIFFERENTIATION BETWEEN LARVAE OF *TIPULA OLERACEA* AND *TIPULA PALUDOSA*

3.1 Isoelectric Focusing

Previous surveys of leatherjacket populations have been unable to distinguish between species of tipulids as the larval stages are morphologically inseparable (eg Chiswell, 1956). Many current workers lack confidence in the reliability of the key characters, anal papillae. The only reliable method of identification is, therefore, to rear larvae through to adults. This method is time consuming and can result in a failure to identify the species because of larval mortality.

Isoelectric focusing has already proved itself as a useful tool in the routine study and management of closely related pest species, eg cyst nematodes (Fleming and Dolan, 1986). The development of such a technique, for the reliable identification of larval *Tipula paludosa* and *Tipula oleracea* recovered from cereals and grassland in Northern Ireland and Scotland, is described below.

3.1.1 Analytical isoelectric focusing

Each life stage was macerated in 50 to 400 µl distilled water depending upon size. Three µl of the liquid macerate were applied directly to the gel surface using an LKB application foil (LKB 18100226). These samples were initially applied to the gel 1.5 cm from the cathode (as suggested by Pharmacia, Sweden) because this produced straight, highly resolved bands. Anodal application became standard practice when it was found to produce interpretable results while significantly reducing the amount of background staining.

3.1.2 Preparation and running of analytical gels

Agarose IEF gels (124 mm x 250 mm x 0.5 mm) consisting of 0.18 g Isogel agarose (FMC), 18 ml distilled water and 0.7 ml Pharmalytes pH 5-6 (Pharmacia), were cast onto a plastic support sheet (Gelbond, FMC) using a capillary gel casting kit (LKB 2117-701). Gels were then stored in a humidity chamber for at least 1 h and a piece of Whatman's No. 1 filter paper was applied to the gel for 1 min just prior to use. Anode and cathode electrode strips were briefly soaked in 0.04 M L-glutamic acid and 1 M NaOH respectively, before being blotted on filter paper until nearly dry. Gels were focused at 6°C using an LKB Multiphor (LKB 2117) and Multitemp apparatus. Prefocusing was carried out for 25 min with voltage output set to 2000 V, current set

to 150 mA and power set to 3 W using a constant power supply (LKB 2197). Sample material was then applied to the gel which was focused for a further 85 min with the power output increased to 7 W.

3.1.3 Staining of gels

Silver staining of focused gels for general proteins followed essentially the method of Vesterberg and Gramstrup-Christensen (1984) but with several modifications. First, gels were fixed for 25 min in 500 ml of a solution containing 0.16 M sulfosalicylic acid, 0.31 M trichloroacetic acid and 0.17 M zinc sulphate. Gels were then washed twice in distilled water for 10 min before being covered with three sheets of Whatman's No. 1 filter paper (the sheet adjacent to the gel being soaked in distilled water) and compressed for 15 min between two glass plates under a 0.5 kg weight. The gel was then dried using a hair dryer before staining. Staining solution A (0.025 M ammonium nitrate, 0.012 M silver nitrate, 0.003 M tungstosilicic acid and 14 ml l^{-1} formaldehyde) was added to staining solution B (0.47 M anhydrous sodium carbonate) in a ratio of 2:1, mixing thoroughly before applying 225 ml to the gel. Staining was allowed to proceed for a maximum of 15 min before being stopped by placing the gel in a 1% solution of acetic acid for 5 min. After drying, gels were stored for future interpretation.

3.2 Identifying unique protein banding patterns for *Tipula oleracea* and *Tipula paludosa*

There are many biochemical techniques available for the separation and identification of proteins. No work of this kind has been previously conducted on tipulids, therefore isoelectric focusing (IEF) was chosen arbitrarily as a starting point to search for proteins unique to one or other of the two leatherjacket species *T. oleracea* and *T. paludosa*.

Use of a general protein silver stain, known to be between 30 and 100 times more sensitive than the commonly used coomassie blue, was adopted from the start. Initially, broad pH range (3-10) pharmalytes were employed with a gradual narrowing of the pH range (5-8; 4-6.5; 5-6) in subsequent gels as areas of interest were identified. Adults of both pest species were collected and cultured from six locations around Aberdeen. Third and fourth instar larvae and pupae and adults of *T. paludosa* were available to compare their banding patterns with those of all stages in the life cycle of *T. oleracea*.

Results and Conclusions

The isoelectric point (pI) of the major protein component extracted from both *T. paludosa* and *T. oleracea* was present in the pharmalyte pH interval 5-6. Both species displayed one protein band with a pI of approximately 5.4, but with that of *T. oleracea* positioned closer to the anode (Fig 3.1; a,b). Though small, the difference between the pI of these bands was still adequate to allow quick species separation. This pH interval also revealed another major protein band with a pI nearer to 5.3 (Fig 3.1; b). For some time the presence of this more anodic band was noted only in *T. oleracea*. However, it was later found to occur (rarely) in *T. paludosa* (Fig 3.1; v). All life stages tested of both *T. paludosa* and *T. oleracea* produced the differentiating bands (Fig 3.1; b-o) and so they could be used to identify eggs, larvae, pupae and adults. Extra protein bands were visible in the pupal and adult samples but they did not interfere with species identification. Two categories of banding pattern variations were encountered on occasion:

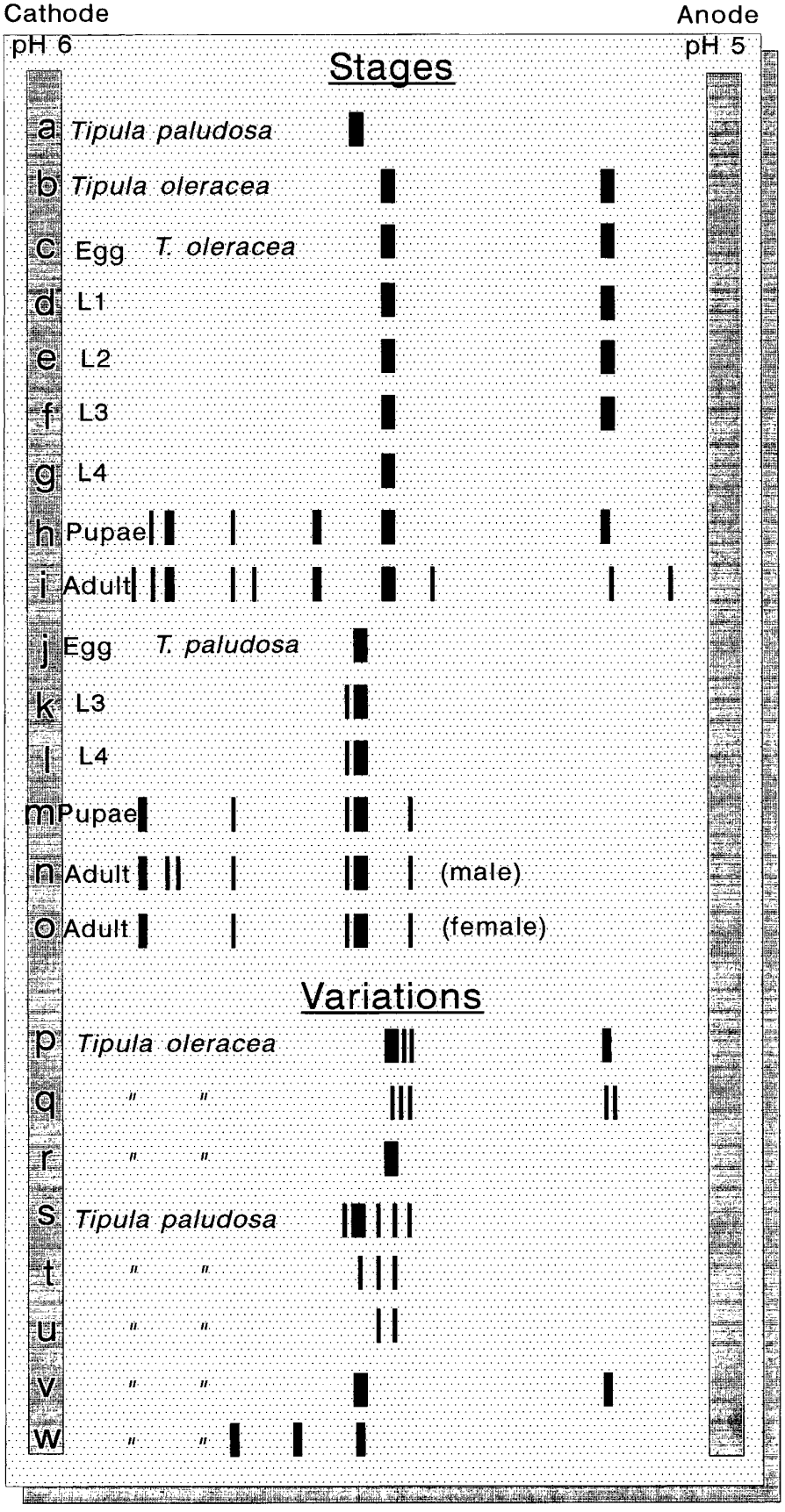
1. Real variation in the protein present in the leatherjacket (Fig 3.1; r, v, w). This was rarely observed and did not affect the position of the differentiating bands.
2. A gradual breakdown of the differentiating bands to produce up to five bands (Fig 3.1; p, q, s-u). If this breakdown was allowed to continue, for example when using the same control material repeatedly, then the differentiating band was eventually lost. This breakdown occurred commonly to some extent in both species.

There were also occasions when samples did not produce protein bands. This was most often caused by inadequate protein being applied to the gel because of small leatherjacket size but was also due to testing of other species which had protein bands with a pI outside the range 5-6.

Isoelectric focusing of tipulid proteins and interpretation of the resulting banding patterns at pH 5-6 is a useful method of resolving the problem of leatherjacket identification. Differences in the constituent proteins of *T. paludosa* and *T. oleracea* can be used to separate the species. The technique has many advantages over other biochemical and genetic methods which could be employed to separate *T. oleracea* and *T. paludosa*. It is fast (approx. 3 hours), can test 88 samples at a time (excluding controls) and is, when used to capacity, relatively cheap.

When using IEF to identify leatherjackets it is recommended that fourth instar leatherjackets of laboratory reared *T. paludosa* and *T. oleracea* should always be used as control samples. Controls should be repeated several times on each gel to allow

Fig 3.1 Banding patterns of the life stages of T. oleracea *and* T. paludosa *showing variations encountered.*



comparison with unknown sample material. A second caveat concerns the gradual breakdown of the differentiating bands at room temperature; material should be stored at -20°C or colder and used immediately upon removal. Live material should be killed by freezing.

3.3 Effect of gut contents on banding patterns

The proteins discovered in Section 3.2 were obtained from the maceration of cultured larvae fed on a diet of dried grass, dried dandelion, bran and rabbit droppings. Banding patterns may reflect, therefore, the proteins present in ingested food or its subsequent breakdown products in the larval gut rather than those of the leatherjackets.

Larvae of the two species were split into two groups; one group was given the food mixture. The second group received no food. Both groups were maintained at room temperature on moist filter paper in Petri dishes. Filter papers were replaced daily for the starved group to limit ingestion of faecal material. After seven days, when no solid material was observed in the guts of starved larvae, specimens from both groups were killed by freezing. Protein samples from starved and fed larvae, along with samples obtained from similarly prepared leatherjacket food were run on a gel and banding patterns compared.

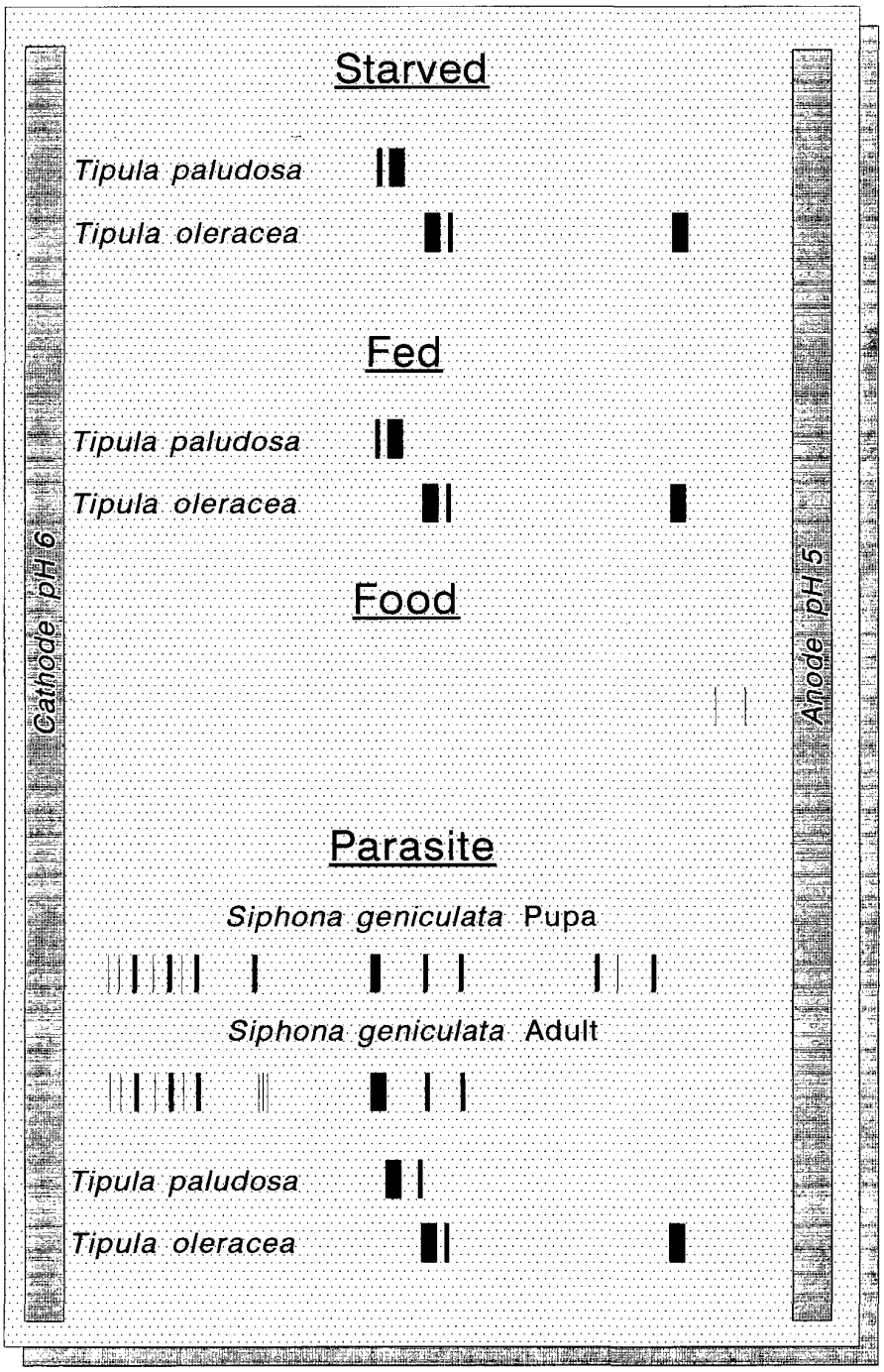
Results and Conclusions

The food mixture produced two very weak bands within the pH range 5-6 (Fig 3.2). These did not coincide with either of the differentiating bands for *T. oleracea* and *T. paludosa*. Banding patterns of starved and fed larvae were identical. Removal of the gut from larvae or starvation of larvae prior to IEF is therefore unnecessary.

3.4 Effect of parasitism on banding patterns

A small proportion of larvae tested contained multiple parasites of *Siphona geniculata* (Diptera: Tachinidae). A single specimen was parasitised by a mermithid nematode, probably *Hexamermith* sp. While it would be difficult to miss such parasitism in its later stages it is possible that parasitised leatherjackets may produce banding patterns that in part reflect the protein of the parasite. Proteins from the parasitoid *S. geniculata* were therefore compared with those of *T. oleracea* and *T. paludosa*.

Fig 3.2 Banding patterns of T. oleracea *and* T. paludosa *(fed and starved) and the food substrate*



Parasitised leatherjackets were cultured and adult *S. geniculata* collected and stored frozen until macerated and focused. Banding patterns of *T. oleracea*, *T. paludosa* and *S. geniculata* were then compared at pH 5-6.

Results and Conclusions

Siphona geniculata samples contained many proteins within the pH range 5-6. Banding patterns of this fly were distinct from those of the leatherjackets. Proteins of similar pI were observed in *T. paludosa* and *S. geniculata* (Fig 3.2), with that of the parasite lying closer to the cathode.

Although the banding pattern of *S. geniculata* contains a band which lies close to the differentiating band of *T. paludosa* the overall configuration of the parasite bands eliminates any difficulty in identification.

3.5 Consistency of the differentiating bands

To determine the extent to which the technique could be used with confidence, specimens of *T. oleracea* and *T. paludosa* were obtained from Spain and The Netherlands, as well as different areas of Scotland and Northern Ireland.

Dead male and female adults of *T. oleracea, T. paludosa* and *T. kleinschmidti* were received from five locations near Santiago, Spain along with dead larvae of *T. oleracea* and *T. paludosa* reared from known adults at Wageningen in The Netherlands. These samples were tested against cultured material from Scotland.

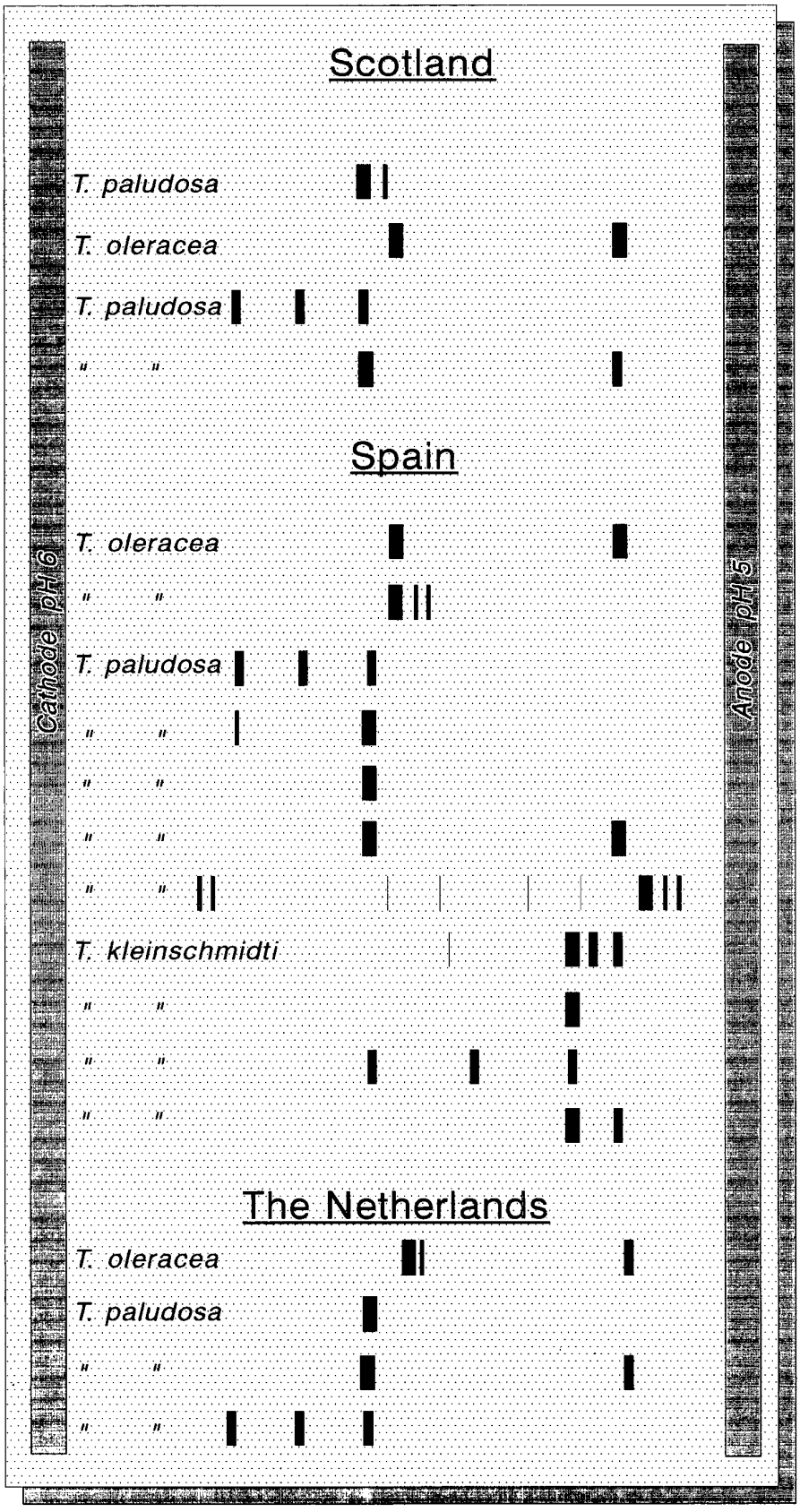
Results and Conclusions

Dutch and Spanish material produced differentiating bands identical to those from Scotland but with 7% of Spanish *T. paludosa* showing a major variation in banding pattern (Fig 3.3). Other Spanish banding pattern variations encountered in *T. paludosa* were also present in specimens from Scotland and The Netherlands (Fig 3.3). These variations did not inhibit the separation of *T. oleracea* from *T. paludosa.*

Material tested from The Netherlands and Spain has confirmed the consistency of the protein banding patterns of the two species revealed in Section 3.2. The differentiating bands identified in that study can be considered reliable for geographically separated populations.

Further variations in general protein banding patterns are likely to be encountered but the reliability of separation of *T. oleracea* and *T. paludosa* using the differentiating

Fig 3.3 Banding patterns of T. oleracea, T. paludosa *and T. kleinschmidti from Scotland, Spain and The Netherlands.*



bands has been demonstrated. Hence, the ability to accurately determine the role of *T. oleracea* larvae in crop damage has been established.

3.6 Broader species comparison

To determine the uniqueness of *T. oleracea* and *T. paludosa* differentiating bands a number of leatherjacket species were compared using IEF. Of particular interest is the banding pattern produced by *Tipula subcunctans* (syn. *czizeki*) which, in the larval form, is visually indistinguishable from *T. oleracea* and *T. paludosa.*

The species is only rarely encountered in the British Isles but is thought to be more common in The Netherlands and Germany. Only three *T. subcunctans* adults were obtained during the course of this project, by breeding unidentified larvae through to adults in Scotland.

Several other species of leatherjackets were collected from grassland and marshy sites in Scotland. These were identified using conventional larval keys (Brindle, 1960). A number of the larvae were reared to adults to confirm their identification. Banding patterns of larval and/or adult specimens of *T. fulvipennis, T. marmorata, T. maxima, T. meigeni, T. subcunctans, T. varipennis, T. vittata* and a Nephrotoma species were compared with those of *T. oleracea* and *T. paludosa* at pH 5-6.

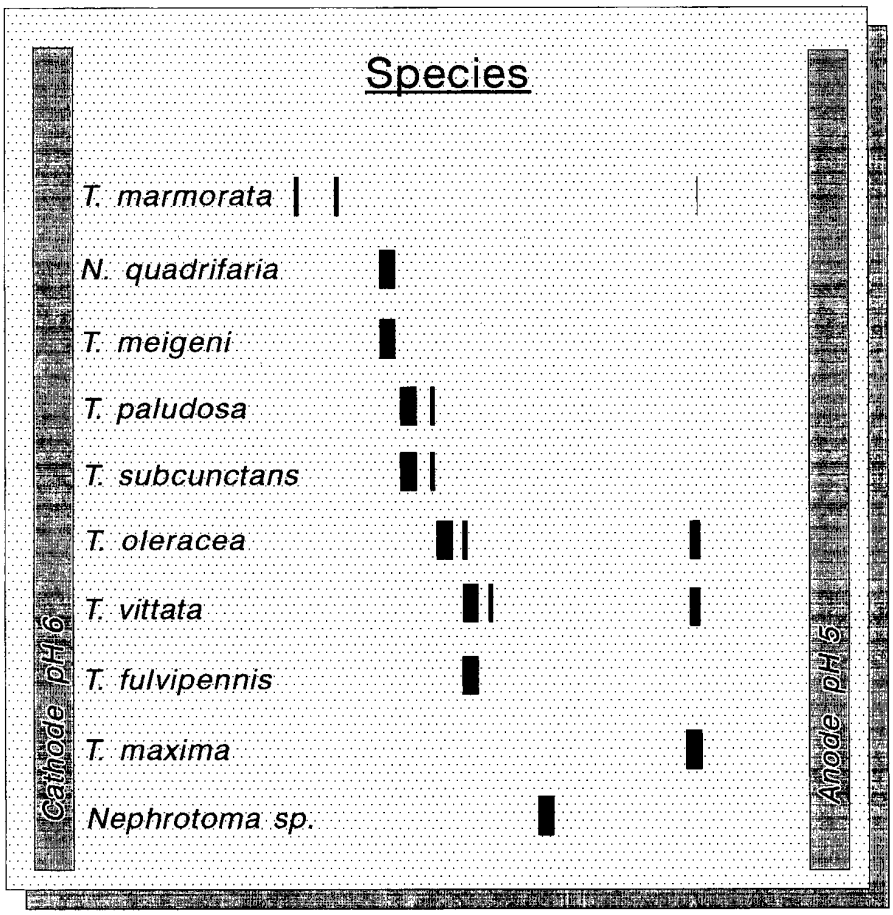
Results and Conclusions

The banding pattern of *T. subcunctans*, based upon three adult specimens, was identical to that of *T. paludosa* (Fig 3.4). All other species tested were readily separated from *T. oleracea* and *T. paludosa.* However, some of these did share common bands which rendered species identification within the pH range 5-6 impossible.

The IEF technique employed can reliably separate *T. oleracea* from all other species tested. Immediate confirmation of the presence of *T. oleracea* in future pest outbreaks is now available.

Tipula subcunctans larvae can be mistaken for *T. paludosa* larvae. However, the low incidence of adults of this species and the fact that it overwinters as eggs (Coe, *et al* 1960) suggests that this problem will only rarely occur and will not be of practical significance to U.K. agriculture.

Fig 3.4 Banding patterns of several leatherjacket species from Ayreshire, Scotland.



3.7 Enzyme-Linked Immunosorbent Assay

3.7.1 Production of antibodies

The major drawback of IEF as a technique for distinguishing between *T. oleracea* and *T. paludosa* is its confinement to the laboratory. In consequence, this project sought to develop a field based technique using an Enzyme-Linked Immunosorbent Assay (ELISA). Such kits are already available for the detection of some viruses (eg ALERT) and a faster, dip-stick technique is currently being developed.

The first stage in this process required the separation and purification of large quantities of the unique distinguishing protein found in *T. oleracea*. The inherent concentrating and resolving powers of preparative IEF make it a suitable method for the one-step purification of this protein. Antibodies can then be produced by immunising rabbits with the protein antigen. This will result in a polyclonal antiserum containing many different antibodies to all the surface structures on the antigen.

A sample consisting of 20 fourth instar *T. oleracea* was macerated in 1% glycine. The macerate was then centrifuged at 3,500 rpm for 10 min to remove particulate matter. The supernatant was dialysed overnight against 1% glycine at 4°C to remove any salt. The sample was again centrifuged, this time at 10,000 rpm for 15 min, to remove particulate matter. The Ultrodex gel used in the separation can hold a maximum of 10 mg ml-1 protein. The sample contained 8 mg ml-1 (total 560 mg in 70 ml), therefore adjustment was unnecessary for the 100 ml gel. Gel preparation consisted of first weighing a 500 ml beaker and then sprinkling 4 g Ultrodex onto 300 ml deionised water. This was left overnight at 4°C allowing the Ultrodex to expand to about 100 ml and settle at the base of the beaker. Excess water was poured off without disturbing the gel. The sample (70 ml) and Pharmalytes pH 5-6 (5 ml) were then added to the gel and the beaker weighed again.

A glass plate (259 x 124 mm) with a silicone rubber rim (internal dimensions 244 x 109 mm) was prepared. This involved cutting six filter paper electrode strips and overlaying three at each end of the gel with the bottom two having been soaked in a 2% solution of Pharmalytes pH 5-6 and the top one soaked in a 7% solution of Pharmalytes pH 5-6. This gave a separation length of 23 cm. The plate was then weighed before and again after addition of the gel slurry mixture. Air bubbles were drawn to the side of the gel and removed with a spatula. A hair dryer was used to dry the gel slurry to its evaporation limit, in this case until 31% of the initial slurry weight was lost. Care was taken to avoid causing rippling of the gel surface.

The gel plate was then positioned on the cooling plate of the Multiphor apparatus with a film of paraffin oil in between. An electrode strip was soaked in 1 M

orthophosphoric acid and placed at the anodic end and another was soaked in 1 M sodium hydroxide and placed at the cathodic end. These strips were cut to length and placed over the existing strips on the gel plate. The electrofocusing lid was then placed over the gel ensuring a good contact between electrodes and electrode strips. The Multiphor was then attached to the power pack and initial settings adjusted to 8W, 1300V and 27mA. Focusing was carried out overnight (15 hours) at 10°C.

The gel was separated into 15 fractions using a fractionating grid. The grid was pushed fully into the gel bed and the gel sections transfered into corresponding elution columns with a spatula. Deionised water was used to elute the proteins. The pH of each sample fraction was then measured before being dialysed against deionised water for 48 hours at 5°C to remove the pharmalytes. Polythylene glycol was sprinkled over the viscous tubing containing the fractions to concentrate the protein. Each fraction was then run on an analytical IEF gel to determine which fraction(s) contained the distinguishing protein for *T. oleracea*. Fractions were then stored at -20°C until work on the production of an ELISA could begin.

Proteins from *T. oleracea*, labelled G, J and M to correspond with the IEF fraction which contained them, were each injected into two New Zealand White rabbits (Table 3.1). Each rabbit received three injections of the protein plus an adjuvant. Each of the six rabbits was then test bled. Red blood cells were removed by allowing them to clot.

Table 3.1 Immunisation protocol for antisera production

			Protein administered (mg) in 0.5 ml of adjuvant		
Protein fraction			G	J	M
Rabbit numbers			1, 2	2, 4	5, 6
Injection date	Location*	Adjuvant†			
24/05/91	IM	FC	0.35	0.5	0.7
10/06/91	IV	FI	0.35	0.5	0.7
24/06/91	VI	FI	0.35	0.5	0.7
13/08/91	IV	FI	0.35	-	-
03/04/92	IV	TM	0.35 (Rabbit 2)	-	-

* IM = intra-muscular, IV = intra-venous

† FC = Freund's Complete, FI = Freund's Incomplete, TM = Titermax

A further booster injection was administered to the two rabbits given protein G as this was considered to be the most promising line of investigation. Nine days later these rabbits were bled and neat (unpurified) antisera were obtained by removal of red blood cells by clotting.

3.7.2 ELISA Protocol

Leatherjackets were macerated in distilled water and the resulting liquid was centrifuged to remove particulate matter before being adjusted to 10 μg ml^{-1} with coating buffer (1.59 g Na2CO3, 2.93 g NaHCO3 and 0.2 g NaN3 in 1 litre distilled water; pH 9.8) using a Pye Unicam PU8800 UV/VIS Spectrophotometer (Philips). A total of 6 ml of antigen were prepared per ELISA plate and 50 μl of leatherjacket antigen was then added to each well. The plate was left to dry overnight in an oven at 37°C. This was fixed with 50 μl methanol which was allowed to evaporate off in an incubator. Each well was blocked for 1 hour using 110 μl of 10% horse serum diluted in PBS extraction buffer (PBS plus 2% PVP; pH 7.4), before being flicked out and the plate dried.

Anti-leatherjacket antibodies were then added to each well in 45 μl PBS extraction buffer. A commercially available anti-rabbit conjugated antibody was added immediately afterwards in 45 μl conjugate buffer (20 g PVP, 2 g ovalbumin made up to 1 litre using PBS-Tween). This mixture was incubated overnight. The plate was then washed five times with PBS, using a Titertek Microplate Washer 120 (Flow Laboratories). The substrate was freshly made by adding 0.01 g substrate to 10 ml substrate buffer (97 ml diethanolamine, 800 ml distilled water and 0.2 g NaN3; pH 9.8, adjusted using HCl). Each well received 50 μl and was incubated at 37°C until a yellow colour became visible in some wells. Plates were then read at 405 nm using a Bio-Kinetics Microplate Reader EL312 (Bio-Tek Instruments).

Results and Conclusions

The seventh and eighth fractions from the cathode contained the differentiating band for *T. oleracea* (Fig 3.1; b). After elution the pH of these fractions was 5.4 and 5.3 respectively. The ninth to eleventh fractions contained the second major band for *T. oleracea* (Fig 3.1; b) and these had pH values of 5.2, 5.1 and 5.0 respectively. These proteins were later injected into rabbits to produce antibodies.

Thus preparative IEF allowed the separation and purification of the differentiating protein band of *T. oleracea.* Whether this band consisted of one or more proteins with

the same pI was unknown. However, antisera were successfully produced for specificity testing.

3.7.3 Effect of altering neat antiserum concentration on its ability to differentiate T. oleracea *from* T. paludosa

In order to proceed with the antisera it was first necessary to determine if species specific antibodies had been produced to any or all of the *T. oleracea* proteins injected into the rabbits. As with all polyclonal antibodies there was the risk that some antigenic determinants were common to proteins from other leatherjacket species.

Six protein antigens, *T. oleracea* proteins G, J and M, whole macerates of *T. oleracea* (O) and *T. paludosa* (P) and a control Keyhole limpet haemocyanin (KLH), were tested against four antisera. As G, J and M and a control virus antisera (V). Proteins G, J and M were coated onto 96 wells (= 1 plate) at concentrations of 5, 15 and 13.3 μg ml-1 respectively while other proteins were coated at 10 μg ml-1. Plates were kept at room temperature and read 45 min, 2 hours and 4 hours after addition of substrate. Neat antisera were serially diluted from 1:50 to 1:64,000 with three replicates of each concentration.

Results and Conclusions

The unpurified antisera exhibited high levels of cross-reactivity (Table 3.2). Differences in reactivity were not significant. Protein G provoked the biggest antibody response overall, from Ab G. Proteins J and M provoked the best responses from their respective antibodies but obtained better reactions with other antibodies. Antisera reactivity was higher against *T. oleracea* than *T. paludosa* in all cases except the control, though not significantly so.

Antisera reaction strength to *T. paludosa* was between 67% and 69% of that obtained for *T. oleracea*.A 1:400 dilution of the neat antisera produced the best differentiation of *T. oleracea* and *T. paludosa* but the specificity of the unpurified antisera G, J and M was too low for immediate use. A further purification step was required to determine if any of these antisera would be able to consistently differentiate between the two species.

Table 3.2 ***Reactivity of neat antisera (1:400 dilution) to leatherjacket proteins. Mean spectrophotometric readings at 405 nm after 4 hours at room temperature.***

Protein Coating concentration (µl ml-1)		Antisera G	J	M	V
KLH	5	0.709	0.374	0.204	0.238
G	15	2.311	0.570	0.324	0.233
J	13.3	0.859	0.817	0.419	0.170
M	10	0.626	0.793	0.553	0.205
O	10	0.521	0.423	0.451	0.199
P	10	0.361	0.284	0.304	0.214

3.7.4 Comparison of purified and neat antisera reactivity with T. oleracea *and* T. paludosa *proteins*

Purified gamma-globulin (IgG) reactivity was compared with neat antisera to determine if two-step purification improved the specificity of the reactions with leatherjacket proteins. In theory most of the antiserum reactivity originates from the IgG fraction and purification reduces cross-reactivity.

Antisera were purified using the two methods described below. No control antiserum was used but, again, a control protein, KLH, was incorporated into the experiment. Coating concentraction was 10 µg ml^{-1} for all proteins and a 1:400 dilution of all antisera was applied. Plates were read after 5 hours at room temperature. There were three replicates of each well except for the neat antisera G and M which had two replicates per test antigen. Well positions were randomised for both coating protein and test antisera.

Five *T. paludosa* larvae were freeze dried and ground to a fine powder. This was sprinkled over 0.5 ml neat antisera (G, J and M) and left for 56 hours at 4°C on a rotating plate. Centrifugation removed particulate matter from the purified antisera. Previously, cross-reacted antisera G, J and M were centrifuged at 3,500 rpm for 30 min. The supernatant was retained and added to an equal volume of distilled water and two volumes of 20% w/v polyethylene glycol (PEG) 6000. The PEG was slowly

added while stirring. This mixture was incubated at 4°C for 30 min and then centrifuged again at 3,500 rpm for a further 10 min. The supernatant was discarded. The pelleted material was resuspended in phosphate buffered saline (PBS). The precipitation with PEG was repeated before the antibody was dialysed against 500 ml PBS at 4°C three times over a 24 hour period. Phosphate buffered saline contained 0.01 M sodium/potassium phosphate (1.36 g l^{-1} KH2PO4, 3.58 g l^{-1} Na2HPO4.12H2O), 0.14 M sodium chloride (8.18 g) and 0.2 g NaN3 and was adjusted to pH 7.4. Purified IgG antibodies (Gp, Jp and Mp) were then adjusted to a concentration of 1 mg ml^{-1} and stored frozen at -40°C.

Results and Conclusions

Purified antisera performed poorly when compared with neat antisera (Table 3.3). Optical density (OD) readings for *T. paludosa* were 28%, 49% and 57% of those for *T. oleracea* using neat antisera G, J and M respectively (cf Table 3.2). Respective figures for the purified antisera were 53%, 63% and 77%. Antiserum reactivity was highest with the protein from which it was produced.

The reactivities to neat antisera were superior to those obtained in Section 3.7.3 yet the only difference in technique was a small variation in protein coating concentration. However, two-step purification of the antisera was detrimental to specificity and resulted in reduced reactivity when compared with the unpurified, neat antisera.

Table 3.3 ***Reactivity of a 1:400 dilution of neat and purified antisera to leatherjacket proteins. Mean spectrophotometric readings at 405 nm after 5 hours at room temperature.***

		Antisera					
Protein ($\mu g\ ml^{-1}$)	Coating	G	Gp	J	Jp	M	Mp
KLH	10	0.289	0.197	0.178	0.128	0.170	0.137
G	10	1.281	1.001	0.614	0.289	0.271	0.221
J	10	0.442	0.397	0.512	0.326	0.366	0.309
M	10	0.432	0.281	0.430	0.308	0.365	0.170
O	10	0.557	0.240	0.325	0.193	0.296	0.175
P	10	0.154	0.126	0.160	0.121	0.169	0.135

Antiserum G reactivity with whole *T. oleracea* proteins was low when compared to reactivity with protein G. This was possibly due to whole *T. oleracea* protein containing only a small fraction of the antigen whereas protein G was pure antigen. Antiserum M showed very poor specificity and, therefore, was discarded at this stage.

3.7.5 Determination of optimum protein coating concentration.

Two concentrations (1:250 and 1:400) of neat antisera G and J were tested against *T. oleracea* and *T. paludosa* protein coating dilutions ranging from 1 μg ml^{-1} to 128 μg ml^{-1}. Three replicates of each well were initially prepared except for antisera J at the 1:250 dilutions where two replicates were set up. Slow reaction speed upon addition of the substrate meant that plates were allowed to develop for 24 hours, of which 5 were at room temperature and 19 at 4°C.

Results and Conclusions

Mean optical density readings (Figs 3.5-3.8) showed that neat antisera had a markedly higher reactivity to *T. oleracea* protein (at concentrations above 8 μg ml^{-1}) than to *T. paludosa*. Antiserum J (Figs 3.5 and 3.6) had a much lower titre than antiserum G (Figs 3.7 and 3.8), although the background reaction with controls (*T. paludosa*) was similar for both antisera. Optical density readings using antiserum G at 1:250 were greater for all but the two highest *T. oleracea* protein concentrations than at 1:400.

Optical density (OD) readings were still increasing even at the highest protein coating concentration tested. Plotting protein concentration against OD produced a sigmoid curve which showed that the rate of OD increase slowed over the final three concentrations (32, 64 and 128 μg ml^{-1}). A coating concentration of 100 μg ml^{-1} and antiserum G at a concentration of 2.5 μg ml^{-1} (ie 1:400 dilution of a 1 mg ml^{-1} stock solution) was concluded to be optimum. At this stage, use of antisera J was discontinued.

3.7.6 Comparison of neat and purified antiserum G and of the two rabbits used to produce this antiserum.

In Section 3.7.5 purified antiserum G performed relatively poorly when compared to the neat antiserum. Two purification steps had been used; cross-reaction and PEG 6000. In this experiment the cross-reaction purification step was removed. Thus, IgG was directly obtained from the neat antiserum using PEG 6000 (see Section 3.3.4).

Fig 3.5 Mean optical density readings of antisera J (1:400 dilution) reactivity to tipulid protein

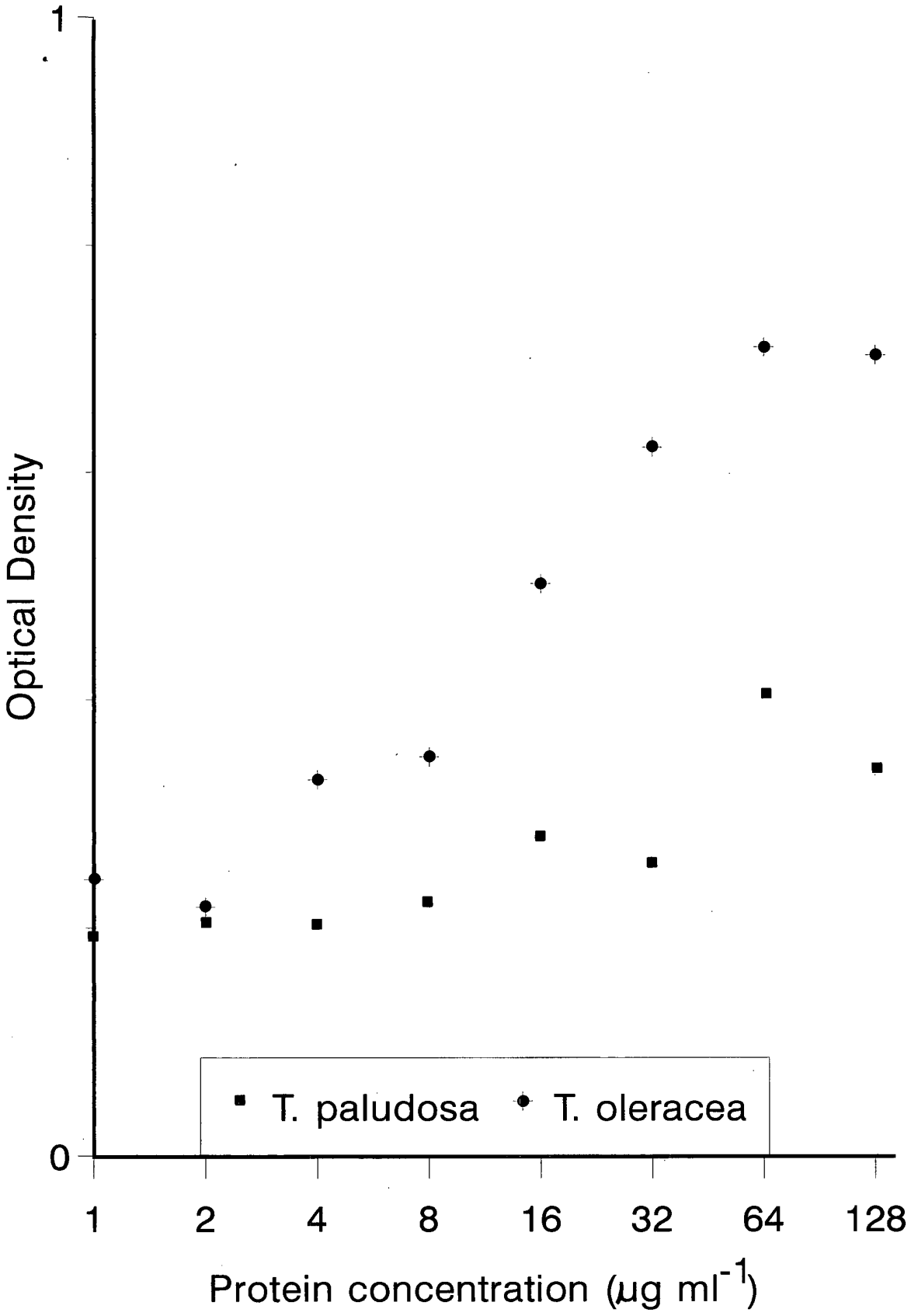


Fig 3.6 Mean optical density readings of antisera J (1:250 dilution) reactivity to tipulid protein

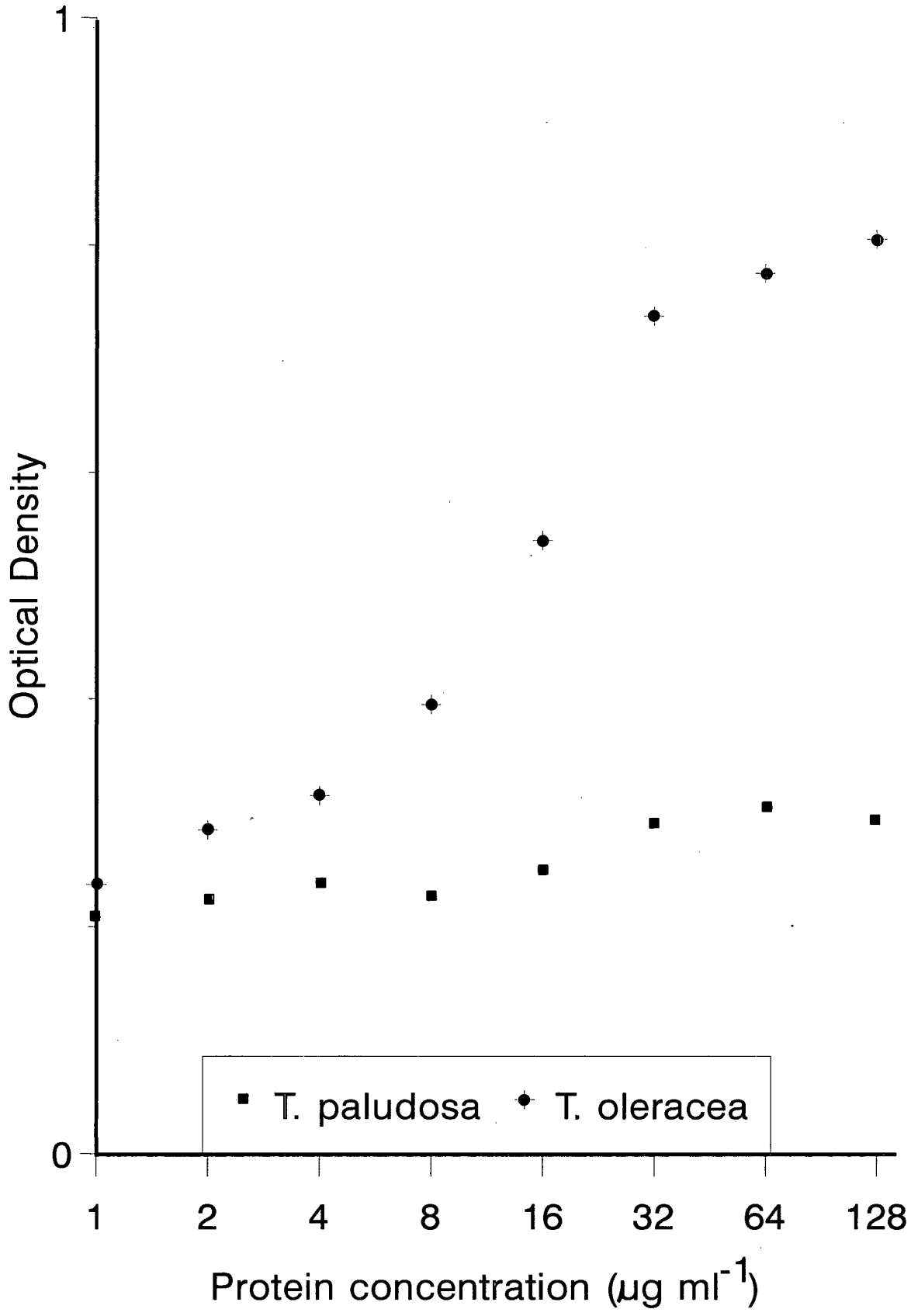


Fig 3.7 Mean optical density readings of antisera G (1:400 dilution) reactivity to tipulid protein

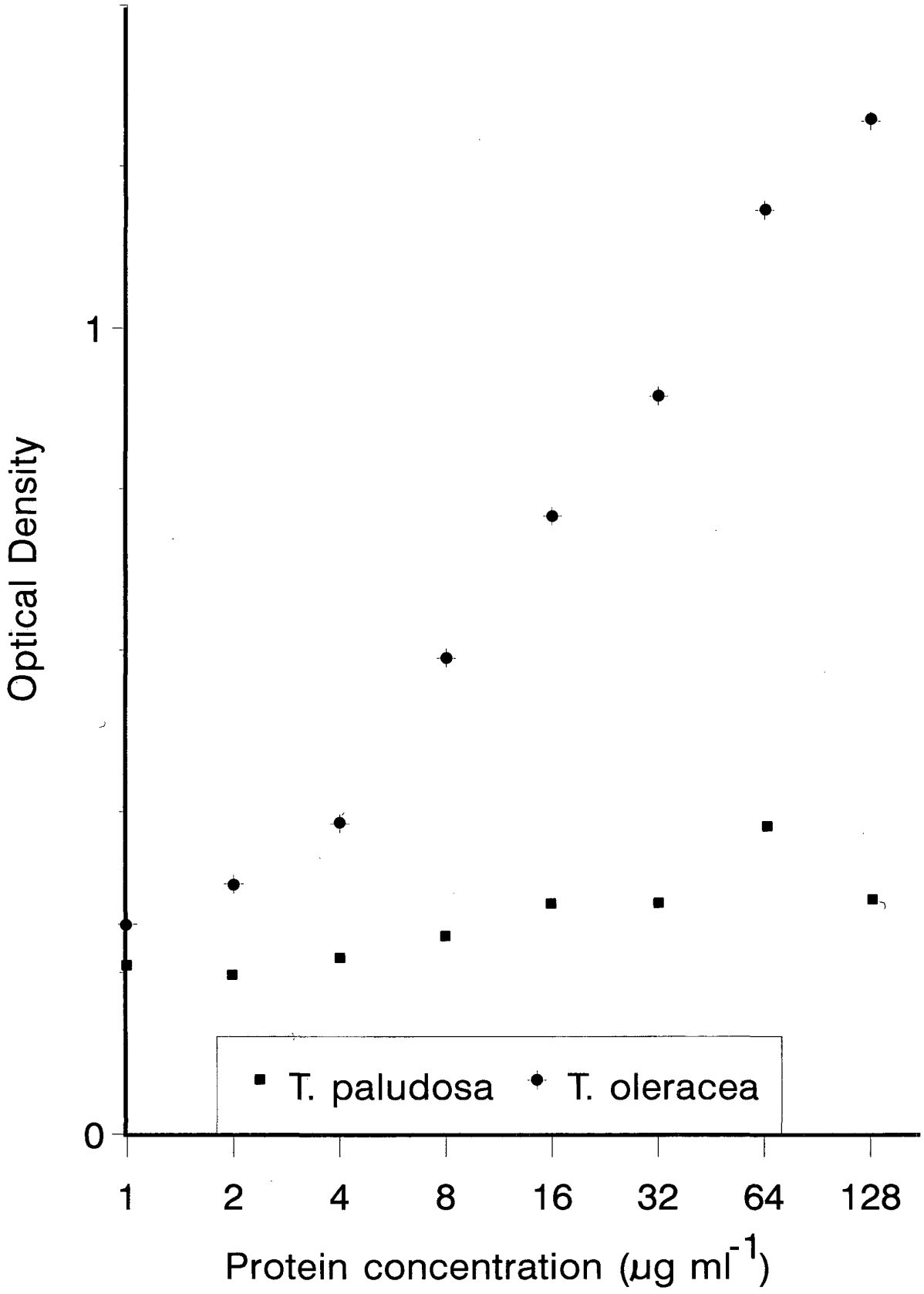
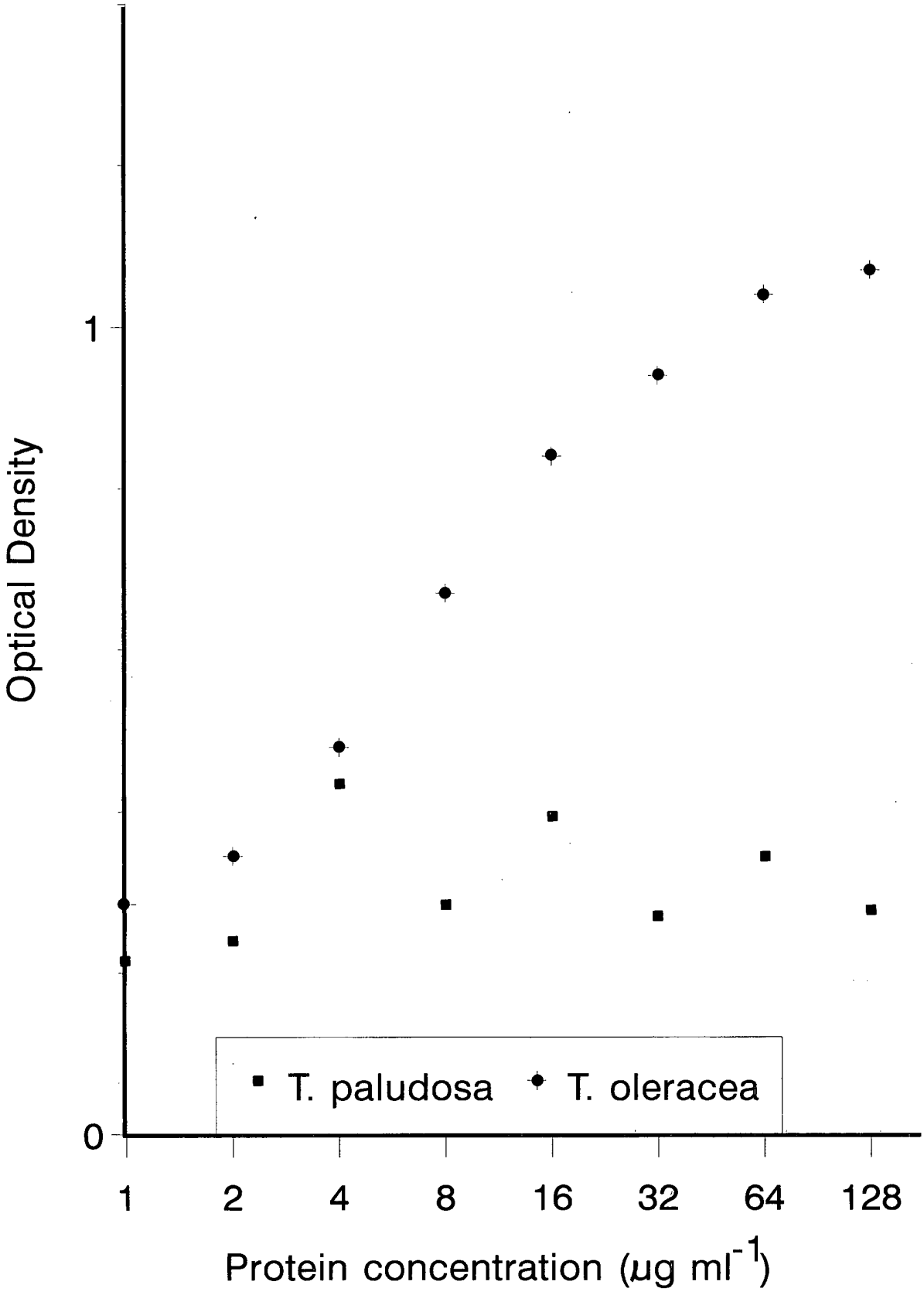


Fig 3.8 Mean optical density readings of antisera G (1:250 dilution) reactivity to tipulid protein



A single plate was coated with *T. oleracea* and *T. paludosa* protein (100 μg ml^{-1}). A 1:400 dilution of each of four antisera (neat and purified As G for Rabbit 1 and 2) was used. Plates were allowed to develop for 24 hours at 4°C before being read spectrophotometrically at 405 nm.

Results and Conclusions

Specificity to the target protein improved with purification of antiserum from Rabbit 1 but not with Rabbit 2 (Table 3.4). Antiserum from Rabbit 2 was less reactive and displayed higher cross-reactivity when purified.

Table 3.4 ***Comparison of optical density using neat and purified antisera G from Rabbits 1 and 2.***

Rabbit	Antisera Type	Protein	
		T. oleracea	*T. paludosa*
1	Neat	0.644	0.288
1	Purified	0.897	0.293
2	Neat	1.103	0.365
2	Purified	0.940	0.405

Neat antiserum from Rabbit 2 had the highest titre against *T. oleracea* protein. Purification had different effects on the two antisera, increasing optical density with Rabbit 1 but decreasing it with Rabbit 2.

3.7.7 Determination of the efficacy of conjugated IgG

The antisera were adequate to differentiate the two leatherjacket species but any resultant test would benefit from improved sensitivity.

Antiserum G from Rabbits 1 and 2, purified by the PEG 6000 method, was used for conjugation with alkaline phosphatase. Plastic multi-well plates were coated with neat and purified antisera from both rabbits. Wells were not replicated.

Purified IgG was conjugated with alkaline phosphatase enzyme (Sigma P5521). This was carried out by resuspending the enzyme in the vial, containing 10,000 diethanolamine (DEA) units. The enzyme was then equally divided between two eppendorf tubes and centrifuged. The supernatant was discarded and the precipitate

dissolved in 1 ml (= 1 mg) of purified globulin. This was then dialysed against 500 ml PBS three times over a 24 hour period. Glutaraldehyde was then added to a concentration of 0.06%. After 4 hours (when a yellow-brown colour may have developed) the mixture was again dialysed three times against PBS to remove the glutaraldehyde. Bovine serum albumin (BSA) was then added to 5 mg ml^{-1} and the conjugated gamma-globulin stored at 4°C.

Purified anti-leatherjacket antibodies were added to each well in 50 µl PBS coating buffer. The coating antisera (purified and neat for both rabbits), at concentrations of 0.1, 1.0 and 10 µg ml^{-1}, was incubated for two hours at room temperature. The plates were then washed three times with PBS using a Titertek Microplate Washer 120 (Flow Laboratories). Excess liquid was removed by flicking the plates. Leatherjacket protein was added at 1 µg ml^{-1} and 10 µg ml^{-1} for *T. paludosa* and 1, 10 and 100 µg ml^{-1} for *T. oleracea* in 50 µl extraction buffer. This was incubated at 4°C overnight. Plates were again washed three times. Conjugated antisera (50 µl per well) was added to wells at dilutions of 1:200, 1:1,000 and 1:2,000. This was incubated for four hours at 37°C. Plates were then washed five times with PBS. The substrate was freshly made by adding 0.01 g substrate to 10 ml substrate buffer. Each well received 50 µl and was incubated at 37°C until a yellow colour became visible in some wells (about 1 hour). Plates were then read at 405 nm using a Bio-Kinetics Microplate Reader EL312 (Bio-Tek Instruments). The well reaction could be stopped by addition of 50 µl 3 M NaOH.

Results and Conclusions

An antiserum coating concentration of 10 µg ml^{-1} was generally too high, resulting in much cross-reaction with *T. paludosa* protein in a very short time span. Optimum *T. oleracea* protein concentration was 100 µg ml^{-1}, the same result as was achieved in Section 3.7.2. The plate coated with purified antiserum from Rabbit 2 failed to produce any colour reaction. Table 3.5 shows the fastest time that combinations of parameters produced a significant difference between OD^{405} readings for *T. oleracea* and *T. paludosa.*

There was little to choose between antisera for the determination of *T. oleracea.* However, where time, conservation of antisera and distance between OD^{405} readings are important the pure antiserum at 0.1 or 1.0 µg ml^{-1} coating and 1:200 or 1:1000 conjugate concentrations gave best results.

Table 3.5 ***Coating and conjugated antiserum concentrations resulting in significant differentiation of* T. oleracea *and* T. paludosa.**

Rabbit	Antisera Type	Conc^{n} µg ml^{-1}	Conj. Conc^{n}	OD read after (hr)	OD^{405} *T. oleracea*	OD^{405} *T. paludosa*
1	Pure	0.1	1:200	1.0	1.010	0.256
		1.0	1:1000	1.0	0.731	0.201
1	Neat	1.0	1:200	1.5	1.414	0.347
		1.0	1:1000	18.5	1.539	0.491
		1.0	1:2000	18.5	1.486	0.413
		10.0	1:2000	1.5	2.237	0.557
2	Neat	0.1	1:1000	1.0	1.934	0.622
		1.0	1:1000	1.0	0.754	0.233

3.7.8 Testing of T. oleracea *and* T. paludosa *using purified antiserum G from Rabbit 1*

Testing of survey material, collected from widely spaced geographic locations and which varied in size (second to fourth instar) was carried out to determine the robustness of the ELISA method as a technique for identifying *T. oleracea.*

Leatherjackets were obtained from a grassland survey of western Scotland in 1992 (Section 5.2.2). The samples had been previously prepared by maceration in approximately 200 µl distilled water for identification by IEF (Section 5.2.2). Protein from each leatherjacket was added to three wells at different dilutions (neat, 1:10 and 1:50) using extraction buffer in all except the neat protein. The ELISA protocol was as for Section 3.7.7, except that 100 µl 10% horse serum was added to each well as an inert blocking protein. This saturated any remaining protein binding sites on the PVC plate after the addition of antigen, but before addition of the conjugated antiserum. Plates were blocked for 1 hour at room temperature. Plates were initially coated at 1, 0.5 and 0.1 µg ml^{-1} and conjugate added at 1:500, 1:1000 and 1:1500.

Results and Conclusions

This experiment failed to differentiate *T. oleracea* (as identified by IEF) from *T. paludosa* by a significant margin. Blank wells actually gave the highest OD reading. A repeat of this experiment produced slightly better results, with control blank wells giving the lowest (but still too high) OD readings and *T. oleracea* the highest (about 1.5 times those of *T. paludosa*).

Results were variable, unspecific and failed to repeat the successes of previous studies. The only new step added to the procedure was blocking with horse serum. This, however, was thought unlikely to be the cause of the reduced antisera specificity.

3.7.9 Comparison of temperature and length of incubation on antisera specificity

This experiment used freshly prepared buffers and followed the ELISA Protocol in Section 3.3.7 except for incubation times/temperatures. Due to the previous loss of antisera specificity a sample statistical test was introduced to differentiate well readings. Six plates were coated overnight at 4°C with purified antisera G at a 1:1000 dilution; three plates each from Rabbits 1 and 2. These plates were then stored at -35°C until required.

Table 3.6 Incubation steps: overnight at 4°C or 3 hours at room temperature

		Incubation times	
Plate Number	Rabbit	Antigen	Conjugate
15.1	1	overnight	overnight
15.2	2	overnight	overnight
15.3	1	overnight	3 hours
15.4	2	overnight	3 hours
15.5	1	3 hours	3 hours
15.6	2	3 hours	3 hours

Tipula oleracea positive standards and *T. paludosa* negative standards were replicated ten times each at 100 $\mu g\ ml^{-1}$. These standards were compared with ten dilutions of three samples (two *T. oleracea* and *T. paludosa*). The protein content of these samples was not measured but they were each diluted using extraction buffer from 1:4 to 1:500. Negative controls contained extraction buffer only. Timing of the incubation steps (Table 3.6) was planned to allow all plates to be simultaneously

started and reactions compared. Confidence limits were set at 99.9% and calculated by: Mean OD ± t x standard error (S.E.) where the S.E. = standard deviation/Sq rt number of replicates and where t has n-1 degrees of freedom, tabulated values.

Limits were calculated for *T. paludosa*. Values for *T. oleracea* greater than this were assumed significantly higher at the 0.01% probability level.

Results and Conclusions

Although nearly every well containing positive and negative controls (whole larval protein at 100 $\mu g\ ml^{-1}$) could be statistically separated this was not true of test material (Table 3.7). Reduced specificity was therefore still a problem.

Table 3.7 ***Percentage of positive wells for each plate 3 hours after addition of substrate, with mean optical density (OD) and standard deviation.***

	Standards		Mean OD		T. oleracea	
Plate Number	*T. oleracea*	s.d.	*T. paludosa*	s.d.	% + ve wells (P<0.001)	Control PBS OD
15.1	2.235	0.2025	1.628	0.2640	90 (10)#	0.640
15.2	0.577*	0.0518	0.465*	0.0436	100 (40)	0.190*
15.3	0.589	0.3913	0.298	0.0511	100 (100)	0.203
15.4	0.475	0.1771	0.301	0.0258	100 (100)	0.255
15.5	0.576	0.0705	0.197	0.0280	100 (55)	0.179
15.6	0.419	0.0362	0.196	0.0182	100 (100)	0.181

* Read after 15 min. OD after 3 hours beyond plate reader limits.

Bracketed figures refer to test protein of whole *T. oleracea*.

The most promising results were obtained by leaving the antigen and the conjugate to incubate for three hours at room temperature with an optimum incubation time after adding substrate of 15 minutes. This resulted in least variation in results and lowest blank well values.

Alteration of the significance level of the test from P<0.001 to P<0.05 made little difference to the results, showing that wells which could not be differentiated were far from being borderline.

The test could only differentiate between *T. oleracea* and *T. paludosa* when protein concentrations were adjusted prior to coating the wells. This process is very time consuming and requires limits for concentration differences which still allow separation of the species to be defined.

3.7.10 Comparison of serial dilutions of T. paludosa *and* T. oleracea *to determine at which protein concentration the species are inseparable*

When protein concentrations of the two species are equal, the ELISA test can separate the species with the aid of simple statistics applied to the OD readings of the wells. However, when many samples are to be tested it is too time consuming to match all the protein concentrations prior to testing. This experiment compared serial dilutions of *T. oleracea* and *T. paludosa* to determine at which concentrations the test fails to separate the species.

Tipula oleracea and *T. paludosa* standards at 100 $\mu g\ ml^{-1}$ were serially diluted 11 times using extraction buffer. Test material, consisting of whole protein macerate from two leatherjackets of each species, was also serially diluted from an initial concentration of 1 $mg\ ml^{-1}$. Conjugate was added at 1:1000. Plate readings were read after 24 hours, being too low to read after 15 minutes and 1 hour.

Results and Conclusions

Tipula paludosa test material produced higher OD readings than did *T. oleracea* in all instances. The experiment was repeated using freshly prepared leatherjacket protein, with the same result.

These results are not easily explained. Optical density readings to determine protein content are one possible source of error. It was noticed that an oxidation process began immediately upon macerating the leatherjackets, producing a brown/black colouration. Such colour differences between the species were often noted with cultured specimens. It is possible that the oxidation process unequally affects OD readings for the two species. This is not, however, thought to provide a full explanation.

3.7.11 Repeat preparation of antisera from Rabbit 2

Due to the failure of the antiserum in experiment 3.7.10 a new antiserum was prepared. Rabbit 2 was given an injection of freshly purified *T. oleracea* differentiating protein with Titermax (see Table 3.1). The PEG 6000 method (Section 3.7.4) was

used to purify IgG, followed by conjugation to alkaline phosphatase (section 3.7.7). Plates were coated with the purified antiserum (1:1000 dilution). Leatherjacket protein was added at 100 μg ml^{-1} and conjugate at 1:1000 and 1:500 dilutions. All incubations were overnight at 4°C. Each well was replicated six times. Samples 5 and 6 and 11 and 12 (Table 3.8) were original whole macerate standards while the preceding pairs of samples were freshly prepared.

Results and Conclusions

Plate readings showed the antiserum was incapable of differentiating the species (Table 3.8). Several further experiments were carried out and involved comparisons of the

Table 3.8 ***Mean plate readings for T. oleracea and T. paludosa using the "new" antiserum.***

	Species	Protein conc^{n} (μl ml^{-1})	Conjugate conc^{n} (dilution)	Mean OD reading	Standard deviation
1.	*T. oleracea*	330	1:1000	0.125	0.0045
2.	*T. oleracea*	330	1:500	0.156	0.0038
3.	*T. oleracea*	100	1:1000	0.128	0.0173
4.	*T. oleracea*	100	1:500	0.170	0.0498
5.	*T. oleracea*	100	1:1000	0.408	0.0419
6.	*T. oleracea*	100	1:500	0.576	0.0261
7.	*T. paludosa*	360	1:1000	0.143	0.0102
8.	*T. paludosa*	360	1:500	0.180	0.0093
9.	*T. paludosa*	100	1:1000	0.151	0.0142
10.	*T. paludosa*	100	1:500	0.188	0.0041
11.	*T. paludosa*	100	1:1000	0.311	0.0438
12.	*T. paludosa*	100	1:500	0.397	0.0529
13.	Blank	-	1:1000	0.146	0.0044
14.	Blank	-	1:500	0.191	0.0075

"old" and "new" antisera, varying coating, protein and conjugate concentrations and incubation times. None yielded different outcomes and it was concluded that the antisera produced were insufficiently sensitive to differentiate *T. oleracea* from *T. paludosa*.

3.8 General Conclusions

Isolectric focussing provided a cheap and robust method for the detection of *T. oleracea* larvae and proved reliable for specimens collected from different geographic regions and of all life stages. The presence of food in the larval gut or parasitoids did not compromise the method.

It was slightly less reliable in identifying *T. paludosa.* Protein bands from this species proved to be identical to those from the closely related *T. subcunctans* so that confusion between the two species could be expected. In practice this is not likely to be a major problem since *T. subcunctans* is infrequently encountered and its life-cycle is not synchronised with that of *T. paludosa.*

Although initial results from the ELISA method had been promising, reasons for the gradual and, ultimately, total failure of the antisera could not be explained. Continuation of this line of study was counterproductive so work on this area of the project was terminated because of the insoluble problem of antisera non-specificity.

The lack of progress in this part of the project did not prevent the achievement of the objectives. Originally, it had been thought that it may have been necessary to develop "dip-stick" technology for the identification of larvae in the field. The opportunities for sampling during the cropping rotation were, however, limited (Section 4) and such a method would have proven redundant.

This is not to say that there would not be advantages to the development of ELISA tests for the identification of leatherjackets because they could provide the additional discrimination to differentiate between *T. paludosa* and *T. subcunctans.* The experience of this study suggests that future work to develop an ELISA test should concentrate on the production of monoclonal, rather than polyclonal, antibodies and should explore different sources of protein than those identified using isoelectric focussing.

4. SAMPLING METHODS

A prerequisite of any pest management programme is the availability of a reliable sampling method to estimate pest numbers. Since *T. oleracea* populations develop in oilseed rape (Section 5) so that damaging numbers may be present when winter cereals are sown, a number of sampling opportunities can be considered. These include within the rape crop, in rape stubble and in the winter cereal crop. Logistical reasons prevented conduct of all experiments at these times so some studies were undertaken in spring barley.

4.1 General experimental details

4.1.1 Use of cultured Tipula oleracea *in experimental plots*

For many of the sampling studies there were insufficient leatherjackets present at the sites for experimental purposes. Laboratory cultured *T. oleracea* larvae were therefore seeded into plots to provide the study population. Larvae were generally released in the late afternoon and left to burrow under the surface. Those that had not done so after 90 min were lightly covered with field soil to provide some protection from bird predation.

Whilst efforts were made to ensure that seeded leatherjackets were of a similar age and size this was not always possible because of constraints on the availability of culture material. Given the range of larval sizes reported from field observations (Section 1), this is not considered to have significantly biased the results of individual studies nor compromised their relevence to problems in winter cereals.

4.1.2 Routine analysis of experimental material

Whenever cultured *T. oleracea* were to be introduced into the experimental plots it was necessary to pre-sample experimental fields to determine which species of leatherjackets were already present (and at what population levels). The standard sampling technique for western Scotland (Section 5.2.2) was employed to pre-sample experimental sites.

These were again sampled upon completion of the experiment, both within the experimental and, occasionally, surrounding areas. Leatherjackets recovered during the experiment were retained and tested using IEF. For complete thoroughness a sub-sample of any cultured larvae that were introduced into plots were also stored separately for IEF analysis using the method described in Section 3.1.

Pre-sampling of fields and testing using IEF allowed species composition and population levels to be estimated. Analysis of banding patterns of specimens recovered at all stages of the experiment ensured the validity of the results. Whilst this constituted an integral part of the

work it was not thought to be of any advantage to include these data. However, it was interesting to note that *T. oleracea* was, on occasion, encountered in oilseed rape stubble in western Scotland.

4.1.3 Experimental design and raw data

Complete details of individual experimental designs and raw data arising from the sampling studies are presented in separate appendices and refered to in the text. Copies of these appendices can be obtained on disk from the senior author.

4.2 Selected sampling methods

4.2.1 Row scratching

In cereals, the technique known as "row scratching" is well established and in widespread use. This involves taking 30 cm drill lengths in a diagonal across a field and hand-sorting through the soil to beneath root depth. Since leatherjackets tend to be concentrated along the drills, this method can give a rapid and reasonably accurate assessment of population levels in a growing crop. It was introduced for use in spring barley in conjunction with a spray threshold of 15 leatherjackets per ten 30 cm drill lengths at a row spacing of 18 cm at the time of seedling emergence. This threshold alters with drill spacing. Where row scratching was used in these studies, samples consisted of ten 30 cm drill lengths per plot.

4.2.2 Wet-sieving

Many leatherjacket surveys in the United Kingdom have been based upon a sample of about 20 soil cores of 10.16 cm diameter and up to 15 cm depth. These are collected in the field and then washed under pressure through a bank of sieves ("wet-sieving") in the laboratory so that the leatherjackets are retained. The origins of this sample size are obscure though the corer, conveniently, is the same size as that used on golf greens. In these studies the wet-sieving process refers to the collection and processing of soil cores 10.16 cm diameter and 8 cm depth.

4.2.3 Brine flotation

A dry-heat extractor was developed by Blasdale (1974) which reduces the amount of time and labour required for extraction of leatherjackets from turf. Sample size is normally 25 cores of 5 cm diameter and 10 cm depth, reflecting the capacity of the apparatus.

Both the wet-sieving and dry-heat methods require laboratory facilities and hence are only available to farmers through intermediaries. In an attempt to obviate this, Stewart and Kozicki (1987) developed an in-field sampling method using saturated brine poured into 10 cm diameter plastic pipes inserted into the pasture turf ("brine flotation"). The salt irritates the leatherjackets and causes them to move to the surface of the soil where they are caught by the solution and float to the surface. The method has not yet found favour in grassland, principally because of the need to transport brine across fields in the winter. In recently cultivated soils, the pipes may be less effective at retaining the brine and so the method may prove not to be an alternative to row scratching in a seed-bed.

In these studies pipes, of 30 cm length, were inserted into soil to a depth of 5 cm and filled to within 5 cm of the top. Observations were made after 15 min. It was necessary to allow for the frequency of pipes emptying through leakage so estimates of leatherjacket population size are based upon the number of pipes that retained brine rather than the number used.

4.2.4 Bait boards

Immobilised leatherjackets have frequently been observed when using methiocarb pellets under wooden boards to monitor slug activity and this suggested a simple sampling method ("bait-board") to detect the presence of leatherjackets and possibly estimate their population density. The boards used in these studies were wooden (although ceramic tiles were also used on occasion) and measured 15 x 15 cm. Before positioning, any herbage present was cut to ground level and 2 g methiocarb (Draza) slug pellets sprinkled over the area to be covered. Stones were placed on boards to prevent them being overturned by birds or blown away. Pellets were neither replenished nor replaced.

Two types of observations were made with bait boards - daily counts with removal of leatherjackets (bait board daily) and counts with no removal (bait board cumulative).
In all studies, the time to observation of leatherjackets under boards is reported relative to the day on which they were first positioned (Day 0) and recorded in units of one day. Where population density estimates are calculated from bait board catches, it is assumed that all larvae recovered from under a bait board previously resided under the are occupied by the board.

4.3 Studies in winter cereals

4.3.1 Sampling methods for Tipula oleracea *larvae in winter barley in autumn (November/December 1990)*

The trial was laid down in a crop of winter barley (var. Puffin) sown on 23 September 1990 in Garden Holm Field (NS 382 230), College Farm, SAC Auchincruive, Ayr in a sandy loam soil. Pre-sampling on 12 November involved 48 soil cores and indicated that no leatherjackets were present at the site.

The experiment was of split plot design with four replicates (Appendix 4.3.1.1.). Main plots, allocated randomly within each block, contained different seeded leatherjacket densities; within each main plot subplots were randomly allocated to different sampling methods. Thus three levels of population density (0.25, 0.50 and 1.00 x 10^6 ha^{-1}) and three sampling methods (row scratching, brine flotation and bait boards) were created. Experimental plots measured 1.0 m x 1.0 m, with a 60 cm guard space between adjacent plots.

Larvae were seeded into plots on 26 November 1990 and left to acclimatise. On 5 December a row scratching sample was taken from each plot. and five plastic pipes for brine flotation were placed at random, upon a length of crop row, along each diagonal of the plot. Immediately following larval extraction by brine flotation the soil circumscribed by each pipe was removed for wet sieving.

Population assessments were also made using four bait boards positioned centrally within each quarter square of the plot. Methiocarb pellets were placed at 14.00 h on 4 December. Bait boards were examined for leatherjackets at daily intervals and numbers recorded and removed. The assessment on Day 1 therefore coincided with the assessment dates for brine flotation and row scratching.

Results and Conclusions

Untransformed data for population assessment by the row scratching method are presented in Appendix 4.3.1.2.

Aggregate numbers of larvae recovered from each of the three seeded population densities, together with these data converted to population densities ha^{-1}, are summarised in Table 4.3.1. The observed population densities were very similar to those of the seeded populations for each of the three treatments.

About 27%, 9% and 8% of the plot area was sampled using row scratching, bait boards and brine flotation respectively. Only 64.2% of the brine flotation pipes retained liquid for the 15 min observation period (Table 4.3.2). The plot area sampled by brine flotation was thus effectively reduced to about 5%.

Table 4.3.1 Comparison of observed population densities, by row scratching, with population densities seeded into plots

Seeded population (x 10^6 ha^{-1})	Observed	
	Total no. larve/treatment	Population (x 10^6 ha^{-1})
0.25	25	0.23
0.50	47	0.44
1.00	97	0.91

Untransformed data for population assessment by the brine flotation method are shown in Appendix 4.3.1.3. Considering only those pipes which retained a surface film of liquid for the entire 15 min observation period, results for each treatment are summarised in Table 4.3.2.

Table 4.3.2 Proportion of pipes from each treatment to retain brine and the efficiency of larval extraction.

Seeded population (x 10^6 ha^{-1})	No. full pipes/40	No. larvae by brine	No. larvae by sieve	Total no. larvae
0.25	25	4	0	4
0.50	27	8	2	10
1.00	25	9	1	10
Totals	77	21	3	24

Of the 120 pipes used in all treatments only 77 (64.2%) retained liquid for the 15 min observation period. From these 77 pipes 21 of the 24 available larvae were obtained by brine, reflecting an extraction efficiency of 87.5%.

Conversion of the numbers of larvae recovered to population equivalents ha^{-1} showed that there was a reasonable correlation between observed and seeded densities for the two smallest leatherjacket populations using brine flotation but that there was a substantial underestimation for the largest population (Table 4.3.3).

In contrast to the row scratching and brine flotation methods, where assessments were recorded at an instant in time, assessments using bait boards were spread over a period of several days.

Table 4.3.3 Comparison of observed population densities, by brine flotation, with population densities seeded into plots (populations as x 10^6 ha^{-1})

Seeded	Observed population	
Population	Brine only	Brine + Sieving
0.25	0.20	0.20
0.50	0.37	0.46
1.00	0.44	0.49

Untransformed data for population assessment by the bait board method appear in Appendix 4.3.1.4. The total numbers of larvae recovered from each treatment on each day of the observation period are summarised in Table 4.3.4.

Table 4.3.4 Total numbers of larvae recovered under bait boards for each treatment on each observation day.

Seeded population (x 10^6 ha^{-1})	Observed population Larvae recorded on each observation day							
	1	2	3	6	8	9	10	Total
0.25	1	5	3	0	2	0	1	12
0.50	1	3	11	2	1	0	0	18
1.00	7	5	30	1	2	0	0	45
Total	9	13	44	3	5	0	1	75
% catch/day	12	17	59	4	7	0	1	

The aggregate catch of leatherjackets increased over time with 88% of total catch attained by Day 3. This time interval is similar to that employed when using the same method to assess slug activity. A three day observation period therefore represents a time scale acceptable to users.

Conversion of the aggregate larval catches by Day 3 to populations ha^{-1} showed that the observed populations related reasonably well to the seeded densities (Table 4.3.5).

The results in Table 4.3.5 suggests that sampling by bait boards provides reliable population estimates across a range of densities. It must be stressed, however, that not only are the estimates derived at a selected instant in time but also that the observed densities are calculated in proportion to the plot area occupied by the bait boards The bait board

Table 4.3.5 Comparison of observed population densities, under bait boards, with population densities seeded into plots (populations as x 10^6 ha^{-1}).

Seeded population	Observed population by Day 3
0.25	0.25
0.50	0.42
1.00	1.17

technique, however, relies on the foraging and feeding behaviour of leatherjackets. Various factors, including weather and soil conditions, are likely to influence larval activity and the probability of individual occurrence under bait boards.

4.3.2 Sampling methods for **Tipula oleracea** *larvae in winter barley in autumn (November 1991)*

The trial was laid down in a crop of winter barley (var. Gauloir) sown on 23 September 1991 in Old Row Field (NS 346296), Monktonhill Farm, Monkton, Ayrshire. The soil type was a sandy loam, tending towards sandy clay loam and preliminary sampling failed to find any leatherjackets.

The experimental design was similar to that in Section 4.3.1. Plots within two blocks only, allocated randomly, were constructed with barriers around their boundaries to constrain larval movement. Barriers consisted of polypropylene lawn edging strip, 16.5 cm wide, dug vertically into the soil to a depth of about 10.0 cm.

Full details of experimental treatments and design are shown in Appendix 4.3.2.1. During the afternoon of 7 November larvae were seeded into the plots at the prescribed population densities

On 19 November a row scratching sample consisting of ten sections of crop row was taken at random from plots. To investigate larval migration from plots two row lengths were also removed from the mid-lines of each of the guard spaces between adjacent plots seeded at the density of 1.00 x 10^6 ha^{-1}. Thus a total of 16 such row lengths, eight from between barriered plots and the same number from between unbarriered plots, was obtained. A random sub-sample of 32 larvae from these exercises was retained for confirmation of identity by the isoelectric focusing technique.

Each plot contained two bait board sampling points, so positioned that each was placed centrally within opposing quarter squares along the same, randomly selected, diagonal of the plot. These boards were randomly allocated to either the bait board (daily) or bait board

(cumulative) method. A wire grid, divided into 36 equally sized squares of 2.5 cm x 2.5 cm, was used on each observation day to monitor the positions occupied by individual larvae under boards for the bait board cumulative.

Methiocarb pellets were placed at 13.30 h on 18 November 1991. At the same time daily thereafter bait boards were examined for leatherjackets.

A random sub-sample of 25 larvae from the bait board (daily) method was collected for confirmation of identity. The 29 larvae which remained on Day 9 of the bait board (cumulative) method were dealt with similarly.

Results and Conclusions

Untransformed data for population assessment by the row scratching method are presented in Appendix 4.3.2.2. Aggregate numbers of larvae recovered from barriered and unbarriered plots for each of the three seeded population densities, together with these data converted to population densities ha^{-1}, are summarised in Table 4.3.6.

The populations observed by row scratching were much lower than those of the seeded populations. The presence of barriers did not influence the results.

The results obtained by row scratching in the guard spaces between adjacent plots seeded

Table 4.3.6 Comparison of observed population densities, by row scratching, with population densities seeded into barriered and unbarriered plots

Seeded population (x 10^6 ha^{-1})	Observed	
	Total no. larvae/treatment	Population (x 10^6 ha^{-1})
0.25 (barriered)	9	0.17
0.25 (unbarriered)	9	0.17
0.50 (barriered)	10	0.19
0.50 (unbarriered)	10	0.19
1.00 (barriered)	29	0.54
1.00 (unbarriered)	15	0.28

at 1.00 x 10^6 ha^{-1} are presented in Appendix 4.3.2.3. The single larva found in the eight row lengths between barriered plots represents a population density of 0.05 x 10^6 ha^{-1}; similarly,one larva was obtained from the eight row lengths between unbarriered plots. Though this low density accounts for little of the discrepancy between observed and seeded densities shown in Table 4.3.6, it does indicate that larval migration may be a factor.

Untransformed data for population assessment by the bait board (daily) method appear in Appendix 4.3.2.4. The total numbers of larvae recovered from barriered and unbarriered plots for each treatment on each day of the observation period are summarised in Table 4.3.7.

Table 4.3.7 Total numbers of larvae recovered under bait board (daily) sampling for each treatment on each observation day.

	Seeded population (x 10^6 ha^{-1})						
	Barriered			Unbarriered			
Day	0.25	0.50	1.00	0.25	0.50	1.00	Total
1	0	1	1	0	1	0	3
2	0	0	0	1	0	0	1
3	0	2	0	0	1	1	4
4	1	2	7	2	3	4	19
5	0	0	0	0	1	2	3
6	0	0	0	0	0	0	0
7	0	0	0	0	0	1	1
8	0	0	0	0	0	1	1
9	0	1	0	0	0	1	2
Total	1	6	8	3	6	10	34

Untransformed data for population assessment by the bait board (cumulative) method are presented in Appendix 4.3.2.5. The total numbers of larvae recorded from barriered and unbarriered plots for each treatment on each day of the observation period are summarised in Table 4.3.8.

The aggregate catch of larvae obtained by the bait board (daily) method (Table 4.3.7) increased with time, although only an occasional individual was recovered after Day 5. The cumulative catch (Table 4.3.8) increased up to Day 6 when it effectively levelled off.

By Day 5, 88% of the total catch made with the bait board (daily) technique had accumulated. The same number of larvae (30) were found on Day 6 using the bait board (cumulative) method. The absolute values for cumulative counts may, however, be illusory since movement of individual larvae from under the bait boards was apparent on Days 7-9 (Table 4.3.8) and noted through changing individual positions. Some leatherjackets may also have been removed as food, either through cannibalism or invertebrate predators.

In Table 4.3.9, the data for aggregate larval catches in barriered and unbarriered plots by Day 5 have been converted to populations ha^{-1} for each bait board treatment.

Table 4.3.8 Total numbers of larvae recorded under bait board (cumulative) sampling for each treatment on each observation day.

	Seeded population (x 10^6 ha^{-1})						
	Barriered			Unbarriered			
Day	0.25	0.50	1.00	0.25	0.50	1.00	Total
1	0	0	1	0	0	0	1
2	1	0	1	0	0	0	2
3	2	1	3	1	0	0	7
4	1	4	5	4	3	3	20
5	3	3	7	3	4	5	25
6	3	4	9	5	4	5	30
7	3	4	8	4	5	4	28
8	3	6	9	5	3	5	31
9	2	6	9	5	4	3	29

Table 4.3.9 Comparison of observed population densities, two bait board methods, with population densities seeded into barriered and unbarriered plots on Day 5.

Seeded Population (x 10^6 ha^{-1})	Observed Population (x 10^6 ha^{-1})	
	Bait board (daily)	Bait board (cumulative)
0.25 (barriered)	0.11	0.33
0.25 (unbarriered)	0.33	0.33
0.50 (barriered)	0.56	0.33
0.50 (unbarriered)	0.67	0.44
1.00 (barriered)	0.89	0.78
1.00 (unbarriered)	0.78	0.56

The Day 5 results obtained by the bait board (cumulative) method are comparable with, though generally somewhat lower than, those acquired by the bait board (daily) method; few of the observed densities by either method differed markedly from those of the seeded populations.

Bait boards apparently provided fairly consistent and accurate estimates of seeded populations across a range of densities, while the results from row scratching appeared to

more seriously underestimate population levels. This poor performance by row scratching was unexpected so that on 27 November all the soil from the two barriered and unbarriered plots seeded at 1.00×10^6 leatherjackets ha^{-1} was recovered and wet-sieved.

In both of the barriered plots the recovery of larvae by row scratching (12 and 17 larvae) and wet-sieving (53 and 47 larvae) is similar and indicates that about 35% of the seeded population was lost from the system. Parallel events occurred in the unbarriered plots but in this case the deficit compared with seeded populations (5 and 10 larvae by row scratching; 29 and 27 larvae by wet-sieving) was in the order of 65%. Though the results presented in Table 4.3.9 appear to suggest that bait boards can provide reasonable assessments of seeded populations, irrespective of the presence of barriers, this was achieved despite the fact that actual populations not only declined during the experiment but may have done so disproportionately with respect to barriered and unbarriered plots. Furthermore there is strong evidence that leatherjackets were relatively mobile within plots and that they may therefore have moved out of or even between plots. Apparently convincing results were achieved despite defects in experimental procedures.

4.4 Studies in oilseed rape

4.4.1 Sampling methods for Tipula oleracea *larvae in winter oilseed rape in spring (April/May 1991)*

The trial was laid down in a crop of winter oilseed rape (var. Libravo) sown on 20 August 1990 into sandy loam soil in Wall Field (NS 356283), Monktonhill Farm, Monkton, Ayrshire. The experimental design was similar to that in Section 4.3.1. Full details of experimental treatments are shown in Appendix 4.4.1.1.

During the evening of 23 April 1991 larvae were seeded into plots at the prescribed population densities. On 8 May the vegetation in plots to be subjected to sampling by the row scratching technique was chopped down and a sample collected.

Each plot contained four bait board sampling points positioned centrally within each quarter square of the plot and randomly divided between the two sampling methods. Methiocarb pellets were placed at 14.00 h on 7 May and observations made at daily intervals thereafter.

Results and Conclusions

Untransformed data for population assessment by the row scratching method are presented in Appendix 4.4.1.2. Aggregate numbers of larvae recovered from each of the three seeded

population densities, together with these data converted to population densities ha-1, are summarised in Table 4.4.1.

Table 4.4.1 Comparison of observed population densities, by row scratching, with those seeded into plots.

Seeded population (x 10^6 ha^{-1})	Observed	
	Total no. larvae/treatment	Population (x 10^6 ha^{-1})
0.25	16	0.15
0.50	20	0.19
1.00	47	0.44

The population density observed by row scratching was much lower than that of the seeded population at all three population densities.

Untransformed data for population assessment by the bait board (daily) method appear in Appendix 4.4.1.3 and for the bait board (cumulative method) in Appendix 4.4.1.4). The total numbers of larvae recovered by the two bait board methods for each treatment on each day of the observation period are summarised in Table 4.4.2.

From Table 4.4.2 it is evident that the aggregate catch of larvae obtained by the bait board (daily) method increased with the passage of time. While it might be expected that the daily running total of larvae by the bait board (cumulative) method would also increase over the observation period, the peak catch (19 larvae) was reached as early as Day 3. The subsequent decline and fluctuations in running totals suggest that larval movement and/or predator activity occurred under the boards.

The above figure of 19 larvae accumulated by Day 3 is similar to the 21 larvae which were collected over the same period for the bait board (daily) method (Table 4.4.2). These 21 larvae represent 68% of the total catch; by Day 5, 87% of the total catch had been achieved.

In Table 4.4.3 the data for aggregate larval catches by Day 3 and by Day 5 for each treatment, using the bait board methods, have been converted to populations ha^{-1}. Population densities observed under bait boards are then compared with population densities seeded into plots.

Both forms of bait board assessment produced similar results for each of the three treatments and consistently underestimated the seeded population densities. This discrepancy was not proportional to the seeded numbers.

The underestimation of the notional population densities by row scratching and bait board methods suggested that leatherjacket dispersal may have occurred. Soil from the guard spaces between plots was therefore examined on 15 May. Two 30 cm row lengths were removed

from each of the mid-lines of guard spaces between adjacent plots seeded at the density of 1.00 x 10^6 ha^{-1}. A total of 18 such row lengths was obtained from which larvae were hand sorted and counted. The results of this analysis are presented in Appendix 4.4.1.5.

Table 4.4.2 Total numbers of larvae recovered under bait boards for each treatment on each observation day.

	Seeded Population (x 10^6 ha^{-1})							
	Bait board (daily)				Bait board (cumulative)			
Day	0.25	0.50	1.00	Total	0.25	0.50	1.00	Total
1	3	1	7	11	1	2	3	6
2	0	1	5	6	1	4	9	14
3	1	1	2	4	1	5	13	19
4	1	0	2	3	2	3	10	15
5	0	1	2	3	1	3	9	13
6	0	0	2	2	1	2	8	11
7	0	0	0	0	3	4	5	12
8	0	0	0	0	3	4	7	14
9	0	0	0	0	3	3	6	12
10	0	0	2	2	3	3	4	10
Total	5	4	22	31	-	-	-	-

Table 4.4.3 Comparison of observed population densities under bait boards with population densities seeded into plots (populations as x 10^6 ha^{-1}).

Seeded Population	Observed Population			
	Bait board (daily)		Bait board (cumulative)	
	Day 3	Day 5	Day 3	Day 5
0.25	0.11	0.14	0.03	0.03
0.50	0.08	0.11	0.14	0.08
1.00	0.39	0.50	0.36	0.25

A total of seven larvae was obtained (equating to 0.15 x 10^6 ha^{-1}). This density was similar to that observed by row scratching in plots seeded at 0.25 x 10^6 ha^{-1} and provided evidence of migration. This factor will have contributed to the apparent underestimation of population densities.

4.4.2 *Sampling methods for* **Tipula oleracea** *larvae in winter oilseed rape in autumn (November 1991)*

The trial was laid down in a crop of winter oilseed rape (var. Falcon) sown in a sandy clay loam on 22 August 1991 in Muirhouse Field (NS 363285), Monktonhill Farm, Monkton, Ayrshire.

The experiment was of randomised block design with four replicates and incorporated a single sampling method, bait board (cumulative), applied to three leatherjacket population densities (0.25, 0.50 and 1.00 x 10^6 ha^{-1}).

Experimental plots measured 1.0 m x 1.0 m, with a 60 cm guard space between adjacent plots. Barriers of polypropylene lawn edging strip, 16.5 cm wide, were dug vertically into the soil to a depth of about 10.0 cm around all plot boundaries.

Full details of experimental treatments and design are shown in Appendix 4.4.2.1. During the afternoon of 18 November 1991 larvae were seeded into the plots at the prescribed population densities.

Methiocarb pellets were placed at two sampling points at 13.30 h on 27 November 1991. At the same time daily thereafter bait boards were examined for leatherjackets. A wire grid, divided into 36 squares of 2.5 cm x 2.5 cm, was used on each observation day to monitor the positions occupied by individual larvae under boards.

The larvae which remained on the last day (Day 9) of the observation period were retained for confirmation of identity by the isoelectric focusing technique.

Results and Conclusions

Untransformed data for population assessment by this bait board method appear in Appendix 4.4.2.2. The total numbers of larvae recorded for each treatment on each day of the observation period are summarised in Table 4.4.4.

Table 4.4.4 Total numbers of larvae recorded under bait boards (cumulative count) for each treatment on each observation day.

Seeded Population (x 10^6 ha^{-1})	Observation day								
	1	2	3	4	5	6	7	8	9
0.25	2	4	3	5	6	5	5	5	5
0.50	3	2	3	3	6	4	4	4	4
1.00	4	6	7	9	7	9	8	11	11
Total	9	12	13	17	19	18	17	20	20

The daily running total of larvae did not consistently increase over the observation period and the data suggest that some leatherjackets either moved, or were taken, from under the boards.

By Day 3 some 65% of the maximum recorded catch had been made and this rose to 95% on Day 5. The running totals for these two observation days were converted to populations ha^{-1} and compared with the seeded population (Table 4.4.5).

Table 4.4.5 Comparison of observed population densities, under bait boards, with population densities seeded into plots (populations as x 10^6 ha^{-1}).

Seeded Population	Observed Population	
	Day 3	Day 5
0.25	0.17	0.33
0.50	0.17	0.33
1.00	0.39	0.39

The population densities observed on Day 3 are, for each of the three treatments, considerably lower than those of the seeded populations. Though by Day 5 observed densities have generally risen, the relationships between observed densities and seeded densities are little improved. Of equal concern, there is no distinction between observed populations for treatments seeded at different densities. Seeded populations are therefore both underestimated and inadequately differentiated.

4.5 Studies in stubbles

4.5.1 Sampling methods for Tipula oleracea *larvae in winter oilseed rape stubble in summer (August 1991).*

The trial site was Wall Field (NS 356 283), Monktonhill Farm, Monkton, Ayrshire. The soil type was a sandy loam. The experiment was laid down in the stubble of winter oilseed rape (var. Libravo) sown on 20 August 1990 and swathed on 28 July 1991. On 29 July the trial area was cleared and a quantity of rape seed was lightly scratched into the soil to ensure an adequate food supply for leatherjackets to be later introduced into the experimental plots. The rape seedlings had emerged by 4 August.

The experiment was of randomised block design with four replicates and incorporated a single sampling method, bait board (cumulative), applied to four leatherjacket population densities (0.25, 0.50, 1.00 and 2.00 x 10^6 ha^{-1}).

Experimental plots measured 1.0 m x 1.0 m, with a 60 cm guard space between adjacent plots. Barriers, consisting of polypropylene lawn edging strip, 16.5 cm wide, dug vertically into the soil to a depth of about 10.0 cm, were inserted around all plot boundaries to constrain larval movement.

Full details of experimental treatments and design are shown in Appendix 4.5.1.1. A delay in crop harvest meant that the larvae intended for use had matured by 8 August and pupation had commenced within the culture. Insufficient larvae remained with which to conduct the proposed programme so the trial proceeded but with seeded densities of 0.50 and 2.00 x 10^6 ha^{-1} omitted from the original scheme.

During the afternoon of 8 August larvae were seeded into the plots of the 0.25 and 1.00 x 10^6 ha^{-1} treatments. In addition, 28 surplus larvae were placed in a vacant plot for the purpose of monitoring the pupation rate of larvae in the trial plots.

On 15 August the entire area of soil, to a depth of about 8.0 cm, was removed from the vacant plot into which the 28 larvae were introduced on 8 August. The mass of soil was then wet-sieved. Of the original 28 larvae only 16 individuals (12 larvae; 4 pupae) remained.

This poor survival rate, combined with the high proportion of pupae within the population, confirmed that pursuit of the experimental objectives would be unprofitable. The trial was therefore abandoned at this stage before the sampling method was set in place.

4.5.2 Sampling methods for **Tipula oleracea** *larvae in winter oilseed rape stubble in summer (August 1992).*

The trial was laid down in the stubble of winter oilseed rape (var. Idol) sown on 12 September 1991 and harvested on 28 July 1992. The site was Barley Hill Field (Irish Grid J242576), Agricultural Research Institute, Hillsborough, Belfast, Co. Down. The soil type was a sandy clay loam of glacial origin and oilseed rape seedlings provided a cover of vegetation over the trial site.

The experiment was of randomised block design with six replicates and five leatherjacket population densities (0., 0.2, 0.4, 0.8 and 1.6 x 10^6 ha^{-1}) seeded on 11 August 1992. A single sampling method was used consisting of a bait board (15 cm x 15 cm) in the centre of a circular experimental plot measuring 0.1 m^2. The boards were positioned after 6 g methiocarb (Draza) pellets were applied (14 August) and observations made three days later (17 August).

Barriers were inserted around all plot boundaries to constrain larval movement. These consisted of steel hoops, 36.0 cm in diameter and 15.0 cm deep, hammered into the soil to a depth of about 7.5 cm. Full details of experimental treatments and design are shown in Appendix 4.5.2.1.

No prior assessment of existing leatherjacket populations was conducted but the introduced population density of 0 x 10^6 ha^{-1} served as a control. A representative sub-sample, consisting of 10 larvae, was retained for confirmation of identity by isoelectric focusing. In addition, a random sub-sample of 10 larvae from the bait board assessment was collected for confirmation of identity.

Results and Conclusions

Untransformed data for larval catches under boards are presented in Appendix 4.5.2.2. In Table 4.5.1 the total numbers of larvae recovered by bait boards have been converted to population densities ha^{-1} and compared with seeded populations.

Larval recovery provided close estimates of the seeded population in terms of the plot area covered by the board (22.5%).

Table 4.5.1 Comparison of observed population densities, by bait board after three days, with population densities seeded into plots.

Seeded population (x 10^6 ha^{-1})	Observed		
	Total no. larvae/treatment		Population (x 10^6 ha^{-1})
0.00	0	(-)#	0.00
0.20	3	(25.0)	0.22
0.40	5	(20.8)	0.37
0.80	9	(18.8)	0.67
1.60	20	(20.8)	1.48

Figures in parentheses are the percentage recovery of introduced leatherjackets

4.5.3 Sampling methods for **Tipula oleracea** *larvae in winter oilseed rape stubble in summer (August 1992).*

The trial was laid down in the stubble of winter oilseed rape (var. Falcon) sown on 22 August 1991 and harvested on 23 July 1992. The site was Muirhouse Field (NS 363285), Monktonhill Farm, Monkton, Ayrshire. The soil type was a sandy clay loam. On 19 August a naturally occurring infestation of leatherjackets was confirmed in the above site. Though of low

density, these larvae presented an opportunity to test sampling methods on natural leatherjacket populations.

A single sampling method, bait (cumulative), was employed in 36 similar plots (2.0 m x 2.0 m with no guard spaces). Results obtained by this method were compared with the known population density confirmed in the site; 48 soil cores were collected on 19 August from within the trial area and wet-sieved for leatherjacket assessment.

Each plot contained a single, centrally placed, sampling point of area 15.0 cm x 15.0 cm and the methiocarb pellets were placed at 11.00 h on 25 August. At the same time for each of the next five days bait boards were examined for leatherjackets. A wire grid, divided into 36 squares 2.5 cm x 2.5 cm was used on each observation day to monitor the positions occupied by individual larvae under boards.

Results and Conclusions

Wet-sieving realised two larvae and one pupa yielding an estimated background population density of 0.08×10^6 ha^{-1} for the site.

A single pupa was observed on all five observation days in the same position in plot 8. A single larva was observed on Day 3 in plot 15 and a single larva was observed on Days 3 and 4 in plot 31; the positions occupied by the latter larvae differed on each day. No other results were obtained.

The maximum catch of three insects occurred on Day 3, yielding an observed density estimate by the bait board (cumulative) method of 0.03×10^6 ha^{-1}. This was similar to that expected from the soil sampling.

All insects from this experiment which were subjected to the isoelectric focusing technique were identified as *T. oleracea*.

4.5.4 Sampling methods for Tipula oleracea *larvae in winter oilseed rape stubble in summer (August/September 1992)*

The trial was laid down in the stubble of winter oilseed rape (var. Falcon) sown on 22 August 1991 and harvested on 23 July 1992. The site was Muirhouse Field (NS 363285), Monktonhill Farm, Monkton, Ayrshire. The soil type was a sandy clay loam. Seedlings from shed rape seed and a few weeds provided a light cover of vegetation over most of the site.

The experiment was of randomised block design with four replicates and incorporated a single sampling method, brine flotation, applied to five seeded leatherjacket population densities, 0.00, 1.23, 2.47, 3.70 and 4.93×10^6 ha^{-1}.

Experimental plots consisted of plastic pipes, each 10.16 cm in diameter and 18.0 cm long, hammered to about half their length into the soil. Each block of treatments was laid out in rows 5.0 m apart, with pipes in each block at 3.0 m intervals.

Full details of experimental treatments and design are shown in Appendix 4.5.4.1. Preliminary sampling on 17 August yielded four leatherjackets from 48 soil cores, equivalent to a density of 0.10×10^6 ha^{-1}.

During the afternoon of 24 August pipes were positioned and leatherjackets were seeded at the prescribed population densities. A representative sub-sample of 39 larvae was retained for confirmation of identity by isoelectric focusing.

Larvae were left to acclimatise within pipes until 2 September when brine was added to each pipeand the num,ber of recovered larvae recorded.

Immediately this extraction the soil circumscribed by each pipe was removed by a soil corer of the same diameter to a depth of about 8.0 cm for wet-sieving. All larvae obtained by brine flotation and sieving were retained for confirmation of identity.

Results and Conclusions

Untransformed data for population assessment by the brine flotation method are presented in Appendix 4.5.4.2. Considering only those pipes which retained a surface film of liquid for the entire observation period, Table 4.5.2 shows the proportions of pipes from each treatment to retain brine and, of these, the proportions of the seeded populations obtained by flotation and by later sieving; from the latter two figures the proportions of the seeded populations lost from the system are derived.

Of the 19 usable pipes from all treatments 16 (84.2%) retained liquid for the 15 min observation period. A single larva, from the highest seeded density, was unaccountable within the system, so reducing the total available larvae from all treatments to 28. From the 16

Table 4.5.2 ***Proportions of pipes from each treatment to retain brine and the efficiency of larval extraction (figures in parentheses as percentages of possible).***

Seeded Population (x 10^6 ha^{-1})	No. full pipes	No. larvae by brine	No. larvae by sieve	No. larvae lost from system
0.00	4/4 (100)	0/0 (-)	0/0 (-)	0/0 (-)
1.23	4/4 (100)	3/4 (75.0)	1/4 (25.0)	0/4 (0.0)
2.47	2/3 (66.7)	4/4 (100.0)	0/4 (0.0)	0/4 (0.0)
3.70	3/4 (75.0)	7/9 (77.8)	2/9 (22.2)	0/9 (0.0)
4.93	3/4 (75.0)	11/12 (91.7)	0/12 (0.0)	1/12 (8.3)
Total	16/19 (84.2)	25/29 (86.2)	3/29 (10.3)	1/29 (3.4)

operational pipes, therefore, 25 of the 28 available larvae were obtained by brine, reflecting an extraction efficiency of 89.3%.

Table 4.5.3 compares observed population densities, by brine flotation and subsequent wet-sieving, with population densities seeded into plots.

Table 4.5.3 Comparison of observed population densities with population densities seeded into plots (populations as x 10^6 ha^{-1}).

Seeded Population	Observed Population	
	1	2
0.00	0.00	0.00
1.23	0.92	1.23
2.47	2.46	2.46
3.70	2.87	3.70
4.93	4.51	4.51

1 from brine flotation 2 from brine flotation and wet-sieving

Though the data are rather meagre, it is evident that the brine flotation technique is operable and robust in oilseed rape stubbles. The extraction efficacy of larvae by brine is very good and the correlation between observed populations and seeded populations is consistently accurate across a range of densities.

4.5.5 Sampling methods for Tipula oleracea *larvae in winter oilseed rape stubble in summer (September 1992)*

The experimental design was similar to that in Section 4.5.4 and was carried out at the same site. Full details of experimental treatments and design are shown in Appendix 4.5.5.1.

Prior to the trial the existing leatherjacket population within the proposed area was assessed. On 17 August 1992 48 soil cores were taken at random from the area and wet-sieved. Four leatherjackets were recovered from the float, indicating that the proposed site was already lightly infested at a density of approximately 0.10 x 10^6 ha^{-1}. Three of these larvae were retained for identification.

During the afternoon of 8 September 1992 pipes were hammered into the soil and immediately thereafter larvae were seeded at the prescribed population densities. A representative sub-sample of 16 larvae was retained for confirmation of identity.

Larvae were left to acclimatise within pipes until 22 September when brine was applied. Immediately following larval extraction by brine flotation the soil circumscribed by each pipe was removed by a soil corer of the same diameter and wet-sieved. All 60 larvae recovered were retained for confirmation of identity.

Results and Conclusions

The three specimens from the leatherjacket infestation present in the site prior to the commencement of the trial were *T. oleracea.* All 16 larvae which formed the sub-sample of those seeded into the plots were *T. paludosa.* All but one of the 60 obtained by brine flotation and sieving were *T. paludosa*; the singleton in this batch was *T. oleracea.*

The preponderance of *T. paludosa* in the recovered larvae was at variance with the known life-cycle of this species (Section 1). The availability of the isoelectric focussing technique and an audit path of specimens showed that the introduction of this species instead of *T. oleracea* resulted from an error in laboratory culture records. The *T. oleracea* found during this experiment could therefore be attributed to a background population already in the oilseed rape stubble.

Untransformed data for population assessment by the brine flotation method are presented in Appendix 4.5.5.2; in these the single *T. oleracea* obtained by flotation has been deleted. Table 4.5.4 shows the proportions of pipes from each treatment to retain brine and, of these, the proportions of the seeded populations obtained by flotation and by later sieving; from the latter two figures the proportions of the seeded populations lost from the system are derived.

Table 4.5.4 ***Proportions of pipes from each treatment to retain brine and the efficiency of larval extraction (figures in parentheses as percentages of possible).***

Seeded population (x 10^6 ha^{-1})	No full pipes		No larvae by brine		No. larvae by sieve		No. larvae lost from system	
0.00	8/8	(100)	0/0	(-)	0/0	(-)	0/0	(-)
1.23	8/8	(100)	8/8	(100)	0/8	(0.0)	0/8	(0.0)
2.47	8/8	(100)	15/16	(93.7)	1/16	(6.3)	0/16	(0.0)
3.70	7/8	(87.5)	14/21	(66.7)	0/21	(0.0)	7/21	(33.3)
4.93	7/8	(87.5)	20/28	(71.4)	0/28	(0.0)	8/28	(28.6)
Total	38/40	(95.0)	57/73	(78.1)	1/73	(1.4)	15/73	(20.5)

Of the 40 pipes used in all treatments 38 (95.0%) retained liquid for the 15 min observation period. 15 larvae, all from the two highest seeded densities, were unaccountable within the system, so reducing the total available larvae from all treatments to 58. From the 38 operational pipes, therefore, 57 of the 58 available larvae were obtained by brine, reflecting an extraction efficiency of 98.3%.

Table 4.5.5 has been constructed to reveal the most relevant features of the results obtained by the brine flotation exercise. In this comparison of observed population densities, by brine flotation, with population densities seeded into plots, all data have been converted to populations ha^{-1}.

Table 4.5.5 Comparison of observed population densities with population densities seeded into plots (populations as x 10^6 ha^{-1}).

Seeded Population	Observed Population	
	1	2
0.00	0.00	0.00
1.23	1.23	1.23
2.47	2.31	2.47
3.70	2.47	2.47
4.93	3.52	3.52

1 from brine flotation 2 from brine flotation and wet-sieving

The results show that the brine flotation technique is operable and robust in oilseed rape stubbles. The extraction efficiency of larvae by brine is excellent. There were losses of leatherjackets from the higher population densities in the experimental system. Reasons for these losses are uncertain but they appear to be density related. In the absence of identifiable predators, cannibalism among leatherjackets appears the most probable explanation.

4.5.6 Sampling methods for Tipula oleracea *larvae in winter oilseed rape stubble in spring (March 1993).*

The trial was laid down in the stubble of winter oilseed rape (var. Falcon) described in Section 4.5.4. A single sampling method, brine flotation, was employed in 25 similar plots in a site containing a natural infestation of leatherjackets. Experimental plots consisted of plastic pipes, each 10.16 cm in diameter and 18.0 cm long, hammered to about half their length into the soil. The experiment was laid out as five rows of plots, with five plots in each row. Plots were spaced at 4.0 m centres. Full details of experimental treatments and design are shown in

Appendix 4.5.6.1. Previous sampling of this site (see 4.5.4 above) had recovered four *T. oleracea* from 48 soil cores.

During late August several species of craneflies, including *T. oleracea* and *T. paludosa*, were active in the site. By 14 December 1992 large numbers of leatherjackets, embracing a range of size and species, were found in soil samples. As most larvae at this time were too small to be readily seen in any of the sampling methods under test within this project, use of this natural infestation was deferred until spring when larvae had become more advanced in size. On 29 March 1993 plastic pipes were positioned in the plots.

Immediately following larval extraction by brine flotation the soil circumscribed by each pipe was removed by a soil corer and wet-sieved. All 38 larvae recovered were retained for confirmation of identity.

Results and Conclusions

Despite the variety of cranefly species active in the area during August and despite also the consequent mixture of larval species and size observed in December, all but one of the 38 larvae recovered by flotation and sieving in this experiment were identified as *T. oleracea* by isoelectric focusing. The reasons for the occurrence and survival of *T. oleracea* in this habitat are unknown but the feature may be significant in the wider contexts of this project (see Section 5.2.1).

Untransformed data for population assessment by the brine flotation method are presented in Appendix 4.5.6.2. Of the 25 pipes used, 24 (96.0%) retained liquid for the 15 min observation period. Ignoring the drained pipe, 30 of the 38 available larvae were obtained by brine, reflecting an extraction efficiency of 78.9%. From the 24 operational pipes the mean number of larvae recovered per pipe was 1.25, representing an observed population density equivalent to 1.54×10^6 ha^{-1}, compared with the estimated population density of 1.94×10^6 ha^{-1} when additional larvae recovered by wet-sieving are included.

The brine flotation technique is, therefore, operable and robust in oilseed rape stubbles. The extraction efficiency of larvae by brine in this natural infestation, though not perfect, provides population assessments accurate enough for most advisory purposes.

4.5.7 Sampling methods for Tipula oleracea *larvae in winter oilseed rape stubble in spring (March/April 1993).*

The trial was laid down at the site described in Section 4.5.4. Twenty-one similar plots were created containing a natural infestation of leatherjackets. Four sampling methods, wet-sieving, bait board (daily), bait board (cumulative) and bait board (cumulative) without pellets (bait board (nil cumulative) were included in each plot.

Plots measured 4.0 m x 4.0 m, with one of the four sampling methods positioned randomly on each of the four corners. No barriers were placed around plots to constrain larval migration but guard spaces, of variable width but never less than 4.0 m, separated adjacent plots. Full details of experimental treatments and design are shown in Appendix 4.5.7.1.

On 29 March 1993 it was confirmed that a natural infestation of *T. oleracea* larvae was present, at a density of 1.94×10^6 ha^{-1}, within this trial area. For the purposes of the current trial this resident population was utilised.

Methiocarb pellets were placed at 12.00 h on 31 March and boards were examined at the same time daily thereafter. At 12.00 h on Day 5 soil was removed to a depth of about 8.0 cm from the 15.0 cm x 15.0 cm single sampling point per plot which had not been previously covered by a board. Each sample was then wet-sieved. Larvae were retained for confirmation of identity by isoelectric focusing.

Results and Conclusions

A total of 66 larvae was recovered from all plots through wet-sieving, equivalent to a population density of 1.40×10^6 ha^{-1}. Of these, 61 larvae yielded protein bands from isoelectric focusing; 57 were *T. oleracea*, three were *Nephrotoma* spp. and one was unknown.

Appendix 4.5.7.2 contains a summary of larval catches per plot by each of the four sampling methods and the total numbers observed under bait boards are presented in Table 4.5.6. Catches per day per plot for each of the bait board methods are given in Appendices 4.5.7.3 - 4.5.7.5. and show considerable spatial variability in populations of leatherjackets within the study area. The peak catch from plots for the cumulative bait board method (Day 5) was significantly correlated ($r=-0.752$; $P<0.05$) with the aggregate catch from the daily bait board method across plots. There were no other significant correlations between sampling methods, indicating that population estimates from bait board methods are unreliable.

Table 4.5.6 ***Total numbers of larvae recovered under bait boards@ on each observation day.***

	Observation Day					
	Day 1	Day 2	Day 3	Day 4	Day 5	Total
Daily	75	57	78	73	41	324
Cumulative	103	152	211	240	282	282
Nil cumulative	13	19	29	11	8	80

@ see text for definitions of sampling methods

From Table 4.5.7 it can be seen that both methods which employed slug pellets yielded similar estimates on each of the observation days, even though individual plot counts were not correlated. Both, however, provided overestimates of the population when compared with the soil sampling, suggesting that counts were strongly influenced by the foraging and feeding behaviour of leatherjackets rather than reflecting the absolute population within the area covered by the boards.

Table 4.5.7 ***Population densities obtained by four sampling methods (populations as x*** 10^6 ha^{-1}***).***

Sampling Method	Observed Population	
	Day 3	Day 5
Bait board (daily)	4.44	6.86
Bait board (cumulative)	4.47	5.97
Bait board (nil cumulative)	0.61	0.17
Wet sieving	-	1.40

4.5.8 Sampling methods for Tipula oleracea *larvae in cereal stubbles in autumn (September/October 1993).*

Rape stubbles are scarce in the vicinity of SAC Auchincruive and so cereal stubbles were used as a substitute to evaluate the probabilities of fluid retention in tubes using the brine flotation technique. Similar trials were undertaken in the stubbles of commercial cereal crops at 16 sites near to SAC Auchincruive, Ayr.

Eight of the sites which had first been tested in spring (see Section 4.6.3) were again selected for the current series of tests. These had followed grass in the rotation. The other eight stubble sites were selected, as far as possible, from the same farms as above but in these the cereal crops followed cereals. Comparison could then be made of the results obtained in the two categories of stubbles sampled in autumn.

As soil type might also influence the performance of brine flotation, soils were classified at each site. Stubbles were selected to provide a wide representation of soil types. No soils were near to field capacity when the brine flotation exercise was conducted.

At each site the method was applied upon a length of crop row at ten widely spaced random intervals along one diagonal of the field. At the end of the observation period each pipe was recorded as being full or drained of brine; provided liquid remained, regardless of depth, the pipe was classified as full.

Cereal harvest dates together with future cropping plans restricted access to some of the sites. Tests were performed on 13 September 1993 at those sites where the farmer intended early ploughing for entry of winter cereals; at most sites, however, experimentation was delayed until 13 or 14 October. Details of the sites are included in Table 4.5.8.

Results and Conclusions

Out of 80 pipes, in the spring test of cereal stubbles that followed grass (Section 4.6.3) 78.8% of the pipes retained brine but when these same soils were later tested in autumn as stubbles (Table 4.5.8 (a)) all pipes supported the brine column.

The presence of buried turf from ploughed in grass may have influenced the performance of brine flotation in spring but by autumn any buried turf was likely to have decomposed. This suggests that the observed improvement in performance is most probably due to the increased soil compaction which occurred during the crop growing season.

From the cereal stubbles following a previous cereal crop (Table 4.5.8 (b)) 96.3% of the pipes functioned for the duration of the observation period. Within the scope of the data, the presence of undersown grasses does not appear to have influenced performance of the method.

Over all 16 sites in this study, 98.1% of the pipes operated successfully in the stubble. The brine flotation method is therefore robust in cereal stubbles across a wide range of soil types.

Table 4.5.8 Retention of brine in pipes in cereal stubbles.

(a) Cereal stubbles:- previous crop grass

Site	Soil type	Date of test	No. pipes full/10	
			autumn	spring#
Kirklandholm	SL	13/9/93	10	8
Strandhead	SL	13/10/93	10	5
Shawwood	SL	14/10/93	10	9
*Burnbrae	SCL	13/9/93	10	8
Broomhill	SCL	14/10/93	10	8
Rowanhill	SCL	14/10/93	10	6
Highpark	CL	13/10/93	10	9
Carbieston	CL	13/10/93	10	10

* = stubble undersown # Sampled spring 1993 - see Section 4.6.3 for details

(b) Cereal stubbles:- previous crop cereals

Site	Soil type	Date of test	No. pipes full/10 autumn
Kirklandholm	SL	13/9/93	8
Auchincruive	SL	13/10/93	10
Shawwood	SL	14/10/93	10
Burnbrae	SSL	13/9/93	10
*Broomhill	SCL	14/10/93	10
Rowanhill	SCL	14/10/93	10
*Highpark	CL	13/10/93	10
*Carbieston	CL	13/10/93	9

SL = Sandy loam
SSL = Sandy silt loam
SCL = Sandy clay loam
CL = Clay loam

* = stubble undersown

4.6 Studies in spring barley

4.6.1 Sampling methods for Tipula oleracea *larvae in spring barley in summer (May/June 1992)*

The trial was laid down in a crop of spring barley (var. Dandy) sown on 22 April 1992 in Barley Hill Field, Agricultural Research Institute, Hillsborough, Belfast, Co. Down (Irish Grid J243576). The soil type was a sandy clay loam of glacial origin.

The experiment was of randomised block design with six replicates and incorporated a single sampling method, bait board (cumulative) using 6 g methiocarb pellets, applied to five leatherjacket population densities, 0, 0.2, 0.4, 0.8 and 1.6 x 10^6 ha^{-1}. Full details of treatments and design are shown in Appendix 4.6.1.1.

Barriers, consisting of steel hoops, 36.0 cm in diameter and 15.0 cm deep, were inserted around all plot boundaries to constrain larval movement. These provided a plot area of 0.1 m^2.

During the afternoon of 29 May 1992 leatherjackets were seeded into the plots at the prescribed population densities. A gas gun scarer was operated within the trial field to deter predation by birds. A representative sub-sample of 10 larvae was retained for confirmation of identity by isoelectric focusing.

Boards were positioned in the centre of the plots on 2 June and an assessment of leatherjackets made on 5 June. Immediately thereafter all soil in all plots was removed to a depth of about 5 cm for wet-sieving.

A random sub-sample of 10 larvae from the bait board assessment was collected for confirmation of identity by the isoelectric focusing technique.

Results and Conclusions

All larvae from this experiment which were subjected to the isoelectric focusing technique were identified as *T. oleracea*.

Untransformed data for larval catches under boards and by sieving appear in Appendix 4.6.1.2.

The boards occupied 22.5% of the plot area. Simplistically, the leatherjacket recovery (18.8 - 29.2%) reflected this fact and indicated that bait boards could be a viable method. Recovery of the residual populations, however, showed that approximately half the seeded leatherjackets were lost during the study (Table 4.6.1). Comparison of the trapped population with the actual population at the end of the experiment showed that the bait boards had, in fact, overestimated the population (Table 4.6.2).

Table 4.6.1 ***Recovery of seeded leatherjackets (x 10^6 ha^{-1}) by bait board sampling and wet-sieving in barriered plots.***

Seeded		Number of leatherjackets		
population	Seeded/plot	Bait boards	Wet-sieving	Total
0	0	0	0	0
0.2	12	3	4	7
0.4	24	7	8	15
0.8	48	9	20	29
1.6	96	22	17	39
Total	180	41	49	90

The most likely reason for this overestimation is the activity of larvae when foraging. Encounter with, and feeding on, the Draza pellets will have immobilised them under the boards giving a greater apparent population density than was the case.

Table 4.6.2 Comparison of seeded, actual and estimated leatherjacket populations (x 10^6 ha^{-1}).

Seeded	Actual	Estimated
0.00	0.00	0.00
0.20	0.12	0.22
0.40	0.25	0.52
0.80	0.48	0.67
1.60	0.65	1.63

4.6.2 Sampling methods for Tipula paludosa *larvae in spring barley in spring (May/June 1992).*

The trials were laid down in commercial spring barley crops at 17 sites near to SAC Auchincruive, Ayr. All crops followed a previous crop of grass. Other than this criterion, no other husbandry or management parameters were applied to site selection.

Soils were not individually classified but they ranged in type from sandy loams to clay loams. Ploughing and seedbed cultivations varied with respect to timings of operations and methods employed. Crop sowing dates and emergence dates were therefore also subject to variation. Rolling of crops postdrilling showed a further range in timings; most were rolled before the start of experimental observations, some were rolled after completion of this work and a few were rolled while the tests were still in progress. At each site two sampling methods, row scratching and bait board (cumulative), were compared.

For experimental purposes the sites were divided into conveniently managed sub-groups of 4, 7 and 6 within which crop sowing dates and crop growth stages were broadly similar. Sampling methods were then applied to each tranche in turn; the sampling programme over all sites extended from 11 May until 29 May. Observations using bait boards occupied seven days for the first two groups of sites and four days for the third. Mid-way through the bait board observation period, but at no consistent timing, the single assessment by row scratching was applied. At each site ten bait board sampling points were laid out at widely spaced random intervals on a line broadly parallel to that from which the row scratching samples were later taken.

All larvae obtained by row scratching from the 17 sites were retained for confirmation of identity by isoelectric focusing. These larvae were taken to be representative of the field populations from which they were obtained.

Six of the sites were resampled using both methods between 2 and 5 June 1992.

Results and Conclusions

Untransformed data for population assessment by the row scratching method at each of the three groups of sites are presented in Appendices 4.6.2.1a-c; similarly, for the bait board method, untransformed data appear in Appendices 4.6.2.2a-c. Aggregate numbers of larvae observed at all sampling points at each site by the bait board and row scratching methods are summarised in Table 4.6.3.

Linear relationship between row scratching counts and numbers under the bait boards explained 5.9, 5.9, 45.1, 46.9 and 49.2% of the observed variation for Days 1, 2, 3, 4 and 7 respectively. The significance of the last three results ($P< 0.01$) was strongly influenced by the results from the Walston (BS) site to the extent that the significance level declined to $P>0.05$ if this site was omitted from the analyses.

There were no significant relationships between numbers obtained by row-scratching and bait board counts in the six resampled sites (Table 4.6.4). June row-scratching counts (Table 4.6.4) were independent of the equivalent May counts (Table 4.6.3).

The lack of relationships between row scratching counts and numbers under bait boards are likely to be due to differences in larval activity between sites. Most leatherjackets recovered during row scratching in May were deep in the soil and associated with ploughed in turf. This may be normal behaviour or could have been induced by the dry weather experienced in the region during the sampling period. The June resampling followed two days of heavy rainfall (31 May/1 June) but with the same general results.

It was assumed that row scratching provided a quantitative estimate of absolute leatherjacket populations. Comparison of results obtained from the May and June samplings at the six sites suggests that this method may not always be as reliable as previously thought.

Among all the larvae obtained by row scratching in the retest a single pupa, at the Carngillan (OS) site, was found. All of these insects and all the larvae from the original tests were identified as *T. paludosa* by isoelectric focusing. The presence of this pupa so early in the season was surprising but, if typical of the population, suggests that many individuals may have been close to pupation. Some degree of prepupal inactivity in the population may also help explain why, at many sites, relatively few larvae were observed under bait boards while catches by row scratching were high.

At all 17 sites during the May assessment by the bait board (cumulative) method the daily running totals of larvae were sustained over the first four days of operation. Daily larval increments were not always achieved but no declines were observed from day to day in the daily running totals. By Day 7, where operated, however, the accumulated totals had declined from previous levels at several sites. Such irregularities have been noted in similar trials during this study but in the current experiment a novel difficulty or deficiency was observed and this may have contributed to the apparent declines.

Table 4.6.3 Aggregate numbers of larvae observed at all sampling points at each site by bait board and row scratching methods.

		Bait boards				
	Row scratching	Day 1	Day 2	Day 3	Day 4	Day 7
(Group 1: 11/5/92 - 18/5/92)						
Corseliehill	1	0	0	0	0	0
Hobsland	2	0	0	0	0	0
Holmes	1	0	1	1	1	0
Ploughland	1	0	0	1	1	0
(Group 2: 18/5/92-25/5/92)						
Carngillan (OS)	5	2	4	4	5	4
Carngillan (SS)	12	0	0	1	3	1
Laigland (Hill)	5	0	2	3	3	2
Laigland (Holm)	1	0	0	0	0	0
Shacklehill	2	0	0	0	0	0
Walston (BS)	18	1	2	7	8	11
Walston (MR)	1	0	1	2	4	5
(Group 3: 25/5/92-29/5/92)						
Belston	4	0	0	0	0	-
Broomhill	3	0	0	0	0	-
Craigs (16 acre)	7	1	1	1	1	-
Craigs (Strip)	2	0	0	0	0	-
Highpark	6	3	5	5	6	-
Langlands	0	0	0	0	1	-

Grubs affected by methiocarb quickly became dehydrated shrivelled and blackened by the combined effects of poison, heat and drought. Many became virtually unrecognisable; it was only with previous knowledge of larval positions beneath bait boards that a meaningful count could be sustained, and then only with difficulty. Almost certainly a farmer or adviser would be unable to identify such grubs if examined only at the end of a 3-4 day period. This feature

may restrict the potential of the bait board (cumulative) method as an effective DIY assessment technique.

Table 4.6.4 Aggregate numbers of larvae observed at all sampling points at six sites resampled by bait board and row scratching methods.

	Row scratching	Bait boards Day 1	Day 2	Day 3
Belston	5	0	0	0
Carngillan (OS)	2	1	1	1
Carngillan (SS)	6	0	0	0
Craigs (16 acre)	3	5	3	3
Highpark	10	1	1	1
Laigland Hill	6	0	0	0

4.6.3 Sampling methods for Tipula paludosa *larvae in spring barley in spring (April/May 1993)*

Trials were laid down in commercial spring barley crops at 18 sites near SAC Auchincruive, Ayr. All crops followed a previous crop of grass. Other than this criterion, no other husbandry or management parameters were applied to site selection.

Ploughing and seedbed cultivations varied with respect to timings of operations and methods employed. Crop sowing dates and emergence dates were therefore also subject to variation. Rolling of crops post-drilling showed a further range in timings; just less than half were rolled before the start of experimental observations, a similar number was rolled after completion of this work and a few were rolled while the tests were still in progress. Soil type was classified at each site.

For experimental purposes the sites were divided into conveniently managed sub-groups within which crop sowing dates and crop growth stages were broadly similar.

At each site the sampling methods brine flotation, row scratching, bait board (cumulative) and bait board (nil cumulative) were compared. No prior assessment was conducted at any site to establish that leatherjackets were present. It was assumed that any infestations would largely consist of *T. paludosa.*

For reasons of convenience, availability and practicability the 18 sites were sub-divided into manageable tranches of 5, 3, 4, 3 and 3, respectively. Sampling methods were then applied to each tranche in turn. Within each group observations using bait boards occupied four successive days. Assessments by row scratching and brine flotation were usually conducted

on the same single day within this period. The complete observation period, over all sites, extended from 21 April until 21 May.

All experimental observations were conducted post-crop emergence. Larvae obtained by row scratching from the 18 sites were retained for confirmation of identify by isoelectric focusing.

At each site the brine flotation method was applied upon a length of crop row at ten widely spaced intervals on a line broadly parallel to that from which the row scratching samples were taken. Ten sampling points for each bait board method were similarly laid out.

Results and Conclusions

Appendices 4.6.3.1a-c contain untransformed data for population assessment by the row scratching method at each of the five groups of sites. Similarly Appendices 4.6.3.2a-c, 4.6.3.3a-e and 4.6.3.4a-e contain untransformed data for the brine flotation, bait board (cumulative) and bait board (nil cumulative) methods, respectively. With the exception of two *Nephrotoma* spp., all larvae subjected to the isoelectric focusing technique were identified as *T. paludosa.*

The results of soil classification and aggregate numbers of leatherjackets recovered by each sampling technique are presented in Table 4.6.4 together with the sampling dates for each group of sites.

Population density estimates derived from row scratching were positively, but weakly, related to those found by brine flotation ($P< 0.05$; 28.8% of variance accounted for) and the bait board (cumulative) method for observations on Day 1 ($P< 0.05$; 27.7% of variance accounted for) and Day 2 ($P< 0.05$; 22.9% of variance accounted for).

The overall figure of 80.6% of pipes retaining brine suggests that this method may be inadequate for taking a sample. A breakdown of percentage retention by soil type yields observations of 70% efficiency in sandy loam, 85.7% in sandy clay loam and 96.7% in clay loam soils. The brine flotation method is therefore less reliable in soils with a higher sand content. This is undoubtedly linked to soil porosity and, with open tilths in seed-beds after cultivation, would make on-farm performance unpredictable and necessitate an operator accepting the need to repeat several pipes to achieve an acceptable sample size.

The relationship between assessments made by the bait board (nil cumulative) method and by row scratching is poor; indeed, on occasion the relationship was negative. The bait board (nil cumulative) method is therefore unreliable for leatherjacket sampling in spring barley.

As stated, sampling of all sites was completed between 21 April and 21 May. While differences in the weather and soil conditions within this period are unlikely to have influenced the quality of the results obtained by the row scratching and brine flotation methods,

Table 4.6.4 Aggregate numbers of leatherjackets recovered by four sampling methods.

			Day 1		Day 2		Day 3		Day 4	
	A	B	C	D	C	D	C	D	C	D
21-25 April 93										
Strandhead 1	3	0 (5)	1	0	6	2	8	3	9	5
Tarcross (Opp) 1	5	1 (4)	6	1	7	2	12	1	13	2
Shawwood 1	5	1 (9)	8	2	15	1	21	2	27	2
Tarcross (Hill) 1	3	0 (8)	3	1	5	1	6	2	7	3
Drumley 1	7	3 (9)	2	1	4	2	7	3	10	1
26-30 April 93										
Langlands 1	3	1 (8)	5	0	7	0	8	0	7	0
Shacklehill 2	8	1 (9)	3	2	5	0	5	1	4	1
Kirklandholm 1	13	1 (8)	3	0	6	1	6	1	6	0
3-7 May 93										
Clune 1	5	1 (5)	2	0	2	0	3	0	2	0
Broomhill 2	26	2 (8)	4	1	10	0	10	0	11	0
Burnbrae 2	18	3 (8)	1	0	1	0	1	0	1	0
Rowanhill 2	10	1 (6)	0	0	1	0	1	0	4	1
10-14 May 93										
B'manston (Down 2	12	5 (10)	4	0	5	0	6	0	8	2
B'manston (Up) 2	29	3 (9)	9	0	10	0	13	1	14	1
McNairston 2	11	2 (10)	1	0	3	0	4	0	4	0
17-21 May 93										
Highpark 3	16	0 (9)	3	0	3	1	3	0	5	1
Carbieston 3	31	6 (10)	21	2	34	5	32	3	52	2
Mossend 2	11	4 (10)	6	0	7	1	8	1	12	0

A - row scratching

B - brine flotation (number of full pipes; n = 10)

C - bait board (cumulative)

D - bait board (nil cumulative)

1 - Sandy Loam

2 - Sandy Clay Loam

3 - Clay Loam

variability in these characteristics may have affected the activity of larvae visiting boards for the bait board (cumulative) method.

When the Group 1 sites were assessed soils were very moist following continuous heavy rain. At all of these sites populations greater than 0.30×10^6 ha^{-1}, the notional economic threshold for spraying, were recorded at some point in time during the observation period for the bait board (cumulative) method. At none of these same sites, however, was this threshold figure attained by the row scratching method. These apparently anomalous results were, however, also reflected in crop damage. Associated with the measured high levels of leatherjacket activity on the soil surface crop damage was also very evident at several sites, notably at Shawwood where the highest counts by the bait board (cumulative) method were recorded. Application of an insecticide spray was considered at this site but rejected, mainly on the basis of the relatively low population assessment achieved by the row scratching method. In the end, no Group 1 site was sprayed even though crop damage was prominent locally in most; fast crop growth rate swung the decision not to spray in cases of doubt. Given the low background densities of leatherjackets, as measured by row scratching, the obvious crop damage and high larval counts under baited boards would appear to be an index of pest activity enhanced by favourable soil conditions. Baited boards almost certainly overestimate larval densities when the pest is active.

In contrast to the Group 1 sites, fields within Groups 2, 3 and 4 were assessed when soil conditions were either much drier or very dry. Only at the Langlands site within the latter ten fields did the assessment by the bait board (cumulative) method, at any point within the observation period, exceed that achieved by row scratching. Furthermore, at only four of these sites did the population obtained by bait boards ever exceed the economic threshold of 0.30×10^6 ha^{-1}. By contrast, eight sites exceeded this figure when assessed by the row scratching method. Assessments by the latter method again proved the more reliable in predicting crop damage. Severe crop damage, exacerbated by dry soil conditions and slow crop growth rates, was observed at several sites. Insecticide sprays were applied at the Burnbrae and Bowmanston (Up) sites. Recommendation was also given to spray the Broomhill and Bowmanston (Down) sites.

Bait boards almost certainly underestimate populations when the pest is inactive in dry soil conditions. Factors other than pest density alone influence the extent of crop damage and the need for insecticide treatments but spray decisions based solely on population assessments by bait boards are liable to be quite erroneous. The Burnbrae site, for example, recorded a maximum population of 0.04×10^6 ha^{-1} by bait boards, yet provided a density of 0.67×10^6 ha^{-1} by row scratching; crop damage was so severe that it was sprayed. Soil conditions were saturated when sampling commenced at the Group 5 sites. Little additional information can be drawn from this small number of samples other than at Carbieston, where of all sites the highest densities were recorded by all sampling methods. In the face of rapidly accelerating

damage by leatherjackets this crop was sprayed with insecticide as a matter of considerable urgency.

In summary, the bait board (nil cumulative) method failed as an assessment technique. The bait board (cumulative) method, dependent upon soil conditions, was liable to greatly overestimate or underestimate larval populations present in the soil. The brine flotation technique was robust in a wide range of recently cultivated soils and provides population estimates closest to those achieved by row scratching. Greater accuracy or consistency with this method might be obtained by increasing the number of sampling points within a field. The requirement to spray crops damaged by leatherjackets largely reflects these observations.

4.7 General conclusions

The studies on sampling methods for *T. oleracea* focussed on three approaches each of which is limited in its applications. Row scratching can be used in cereal crops where the growing plants provide the food source so that leatherjackets are concentrated along crop rows. In oilseed rape, however, fallen leaves are likely to be a food source over much of the growing season (see Section 6.2). Any larvae will be dispersed within the crop and row scratching would underestimate their number.

Brine flotation proved to be robust and efficient when used on compacted soils such as stubbles. In recently cultivated soils, however, retention of solution was a problem and this is likely to frustrate most users. An exception may occur with heavier soils but there are insufficient data to make recommendations on this basis.

Results from bait board methods were highly variable and greatly influenced by leatherjacket activity; there were occasions of under and overestimation of population levels. Nevertheless, if the threshold climatic and soil conditions for leatherjacket activity were understood this method could have a role as an easy, cheap means of detecting the presence of larvae to determine to which fields more labour intensive methods should be applied.

Where population estimates were linked with perceived damage (e.g. Section 4.6.3), there was an implication that bait board counts may reflect the potential for damage, suggesting that economic thresholds should encompass activity as well as population size.

At the start of this project the timing of sampling was unknown so that studies were undertaken in winter cereals in the autumn, growing oilseed rape and stubbles. Sampling within a rape crop after the autumn is problematic because of the height and density of the canopy and autumn sampling of cereals may be too late to avoid damage or to gain sprayer access to the land. Given that a preceding crop of oilseed rape is the dominant agronomical factor influencing leatherjacket numbers in winter cereals (Section 5), the critical time for sampling is after rape harvest and before seed-bed preparation. At this time, brine flotation will provide a usable method for farmers and advisers.

5. PREDISPOSING FACTORS

5.1 Survey of growers

In the autumn/winter of 1990/91 a questionnaire was sent to farmers, advisers and crop consultants who had reported leatherjackets in winter cereals. The questionnaire was designed to be simple to complete and, in consequence, used imperial rather than metric units. A total of 250 were sent out.

Results and Discussion

Sixteen questionnaires were returned from farmers in Aberdeen, Gordon, Banff and Buchan and Kincardine and Teeside. An additional 15 were completed from clients consulting the Crop Clinic at SAC Aberdeen. Growers were not always able to answer every question so the results are presented as percentages of answers.

Winter wheat comprised 90% of the crops attacked (Fig 3.1) and 84% of attacked crops were preceded by oilseed rape (Fig 5.2). The majority of attacked crops were sown in late September or October (Fig 5.3) but only 22% had been rolled (Fig 5.4). Larvae were found feeding on plants from October onwards (Fig 5.5) and these ranged in size from second to fourth instar though in most crops a range of instars were observed (Fig 5.6). Thirty percent of population estimates lay between five and twenty per square foot (929 cm^2) (Fig 5.7), equivalent to 0.54-2.15 x 10^6 leatherjackets ha^{-1}. In only 16% of cases (Fig 5.8) was damage described as "light" and this compares with 26% of crops with a reported population less than 2 per square foot (Fig 5.7). The previous crop had been harvested (if oilseed rape or winter wheat) in all cases by early September 1989 (Fig 5.9) but 74% had been sown by late August 1988 (Fig 5.10). Most of the damage was reported from loam or clay soils (Fig 5.11) with a high moisture content (Fig 5.12).

An important *caveat* in interpreting these results is that the observations are subjective. Nevertheless, the pooled responses do provide insight into agronomic factors that are influencing the occurrence of leatherjackets in winter cereals.

Although winter wheat is grown more commonly than winter barley in north-east Scotland, the ratio of cropping area (2:1) is considerably less than the reported ratio of damaged crops (9:1) shown in Fig 5.1. Thus it is reasonable to conclude that winter wheat is more at risk from leatherjacket damage. Present knowledge suggests that this is more likely to be a function of rotation than host crop susceptibility.

The link with a preceding crop of oilseed rape was substantiated by these farmers' reports and this, with the range of leatherjacket instars found, suggests that *T. paludosa* was not the

Questionnaire responses about leatherjacket damage

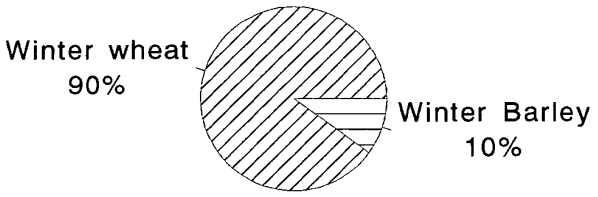


Fig 5.1 Damaged Crop

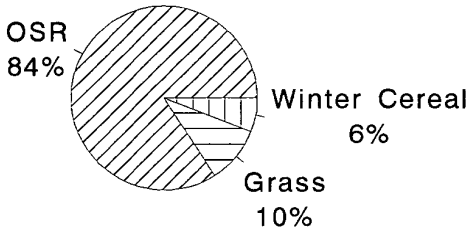


Fig 5.2 Previous Crop

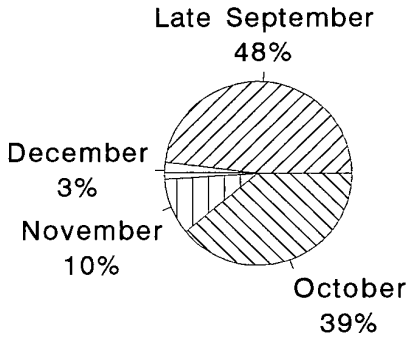


Fig 5.3 Date Damaged Crop Sown

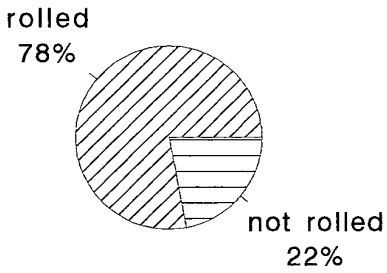


Fig 5.4 Seed-bed Preparation

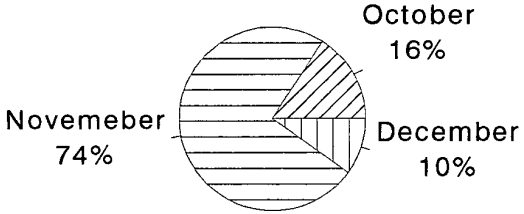


Fig 5.5 Time of Damage

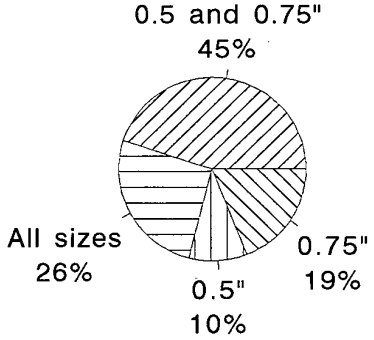


Fig 5.6 Size of Larvae

Questionnaire responses about leatherjacket damage

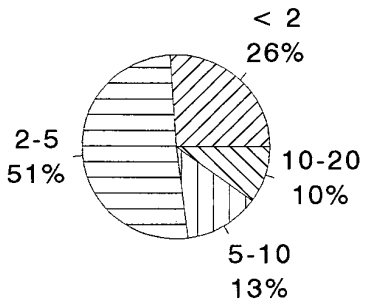


Fig 5.7 Numbers per Square Foot

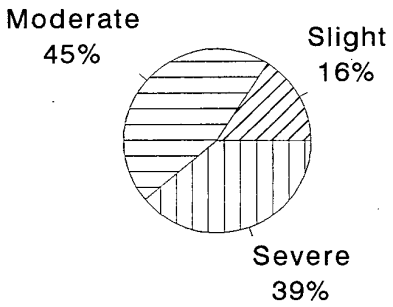


Fig 5.8 Extent of Damage

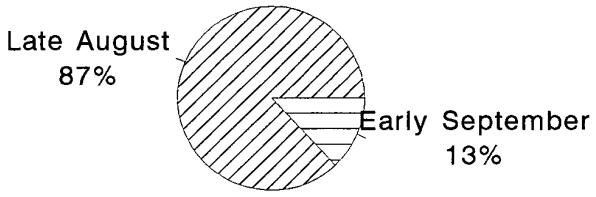


Fig 5.9 Harvest of Previous Crop

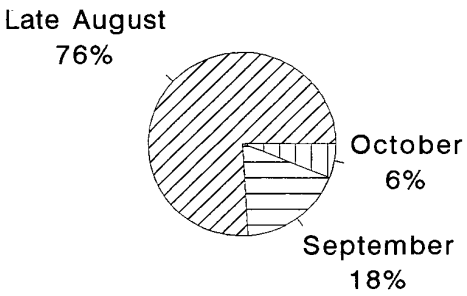


Fig 5.10 Date Previous Crop Sown

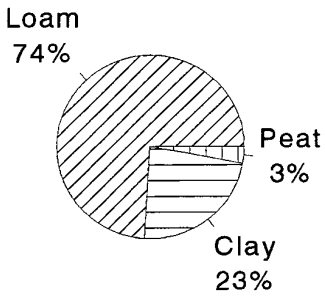


Fig 5.11 Soil Type

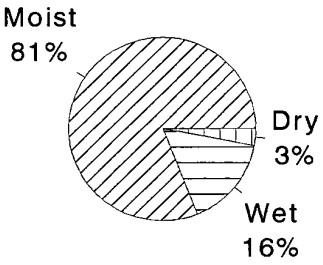


Fig 5.12 Soil Moisture

major problem. It is possible, however, that this species did occur in those crops following grass in a rotation.

There appeared to be an increased risk when the rape crop was harvested late (by local standards) and followed by a relatively early sowing of the cereal; a short interval between crops may favour the survival of leatherjackets already present in the soil.

The majority of crops in which leatherjackets were found had not been rolled. It is known that rolling will reduce damaging attacks on spring barley and it has even been suggested as a control method for *T. paludosa* in grassland (Kell and Blackshaw, 1988). It probably does not actually kill larvae, rather it impedes access to plants by capping the soil.

The most common soil in north-east Scotland for growing winter cereals is loam. The dependence of *T. oleracea* eggs and larvae on moisture for survival (Meats, 1967; 1970; 1972) makes it unsurprising that leatherjackets were mainly associated with wetter soils (Fig 5.12). Further field observations frequently associated the presence of leatherjackets with slugs in winter cereal crops and these pests are well documented as favouring moisture retentive soils.

Many of the farmers involved in this study had previously noticed similar damage to crops following rape but had attributed it principally to slugs. Where leatherjackets had been seen, it had been assumed that it was normal to find them in cereal crops because of experience with spring barley. On this basis, leatherjackets at low population densities were assumed below an economic threshold and treated as inconsequential. Experience of leatherjacket feeding in grassland, however, has shown that low temperatures and slow plant growth over the winter enables relatively few active larvae to cause significant damage (Blackshaw, 1985).

5.2 Distribution of *Tipula paludosa* and *Tipula oleracea*

5.2.1 Occurrence in winter wheat and oilseed rape

Fifteen fields of oilseed rape and winter wheat were chosen for investigation during the 1991/92 growing season in Banff, Buchan, Gordon and Kincardineshire. The fields were paired so that an oilseed rape and a winter wheat field was chosen from the same farm. All of the fields examined were situated in, and around, sites which had previously experienced problems with *T. oleracea*.

The crops were examined on three separate occasions in early November, March and immediately after harvest. On each occasion, ten 1 m drill lengths per field were sampled. Soil was taken from below root depth and approximately 5 cm either side of the drill.

On the first sampling date (November) the soil was put into bags and taken back to the laboratory where it was sifted and handsorted and the number of larvae recorded. On the second and third assessment dates ten 1 m drill lengths were dug up and laid out onto white

sampling trays. The soil was sorted through by hand and the number of larvae found were recorded. The eggs and larvae were identified on each occasion using the method described in Section 3.1.

Results and Conclusions

All leatherjackets were identified as *T. oleracea.* On four of the farms no larvae were found in either the wheat or the oilseed rape crops, ten had leatherjackets in the oilseed rape crop but not in the wheat crops but only one farm had larvae in the winter wheat but not in the oilseed rape. This difference in occurrence was statistically significant (sign test; $P< 0.05$) and provides evidence that *T. oleracea* occurs more frequently in oilseed rape than in winter wheat.

Both the number of fields yielding leatherjackets and the total number of larvae recovered from each crop (Table 5.1) changed through the growing season. The increase for both winter wheat and oilseed rape counts between November 1991 and March 1992 can be accounted for by increased individual size and concomittant probability of recovery. Between March and September 1992 the probability of recovering larvae from oilseed rape doubled (from 0.33 to 0.73) whereas that for winter wheat remained unchanged. The number of larvae recovered from the oilseed rape crops, however, increased by 757%. There was therefore a strong suggestion of a population increase in the oilseed rape that did not occur in the winter wheat.

Table 5.1 ***The occurrence of* Tipula oleracea *larvae in oilseed rape and winter wheat crops (n=15).***

	Fields with larvae		Numbers of larvae	
Date	Rape	Wheat	Rape	Wheat
Nov 1991	3	0	3	0
Mar 1992	5	3	7	3
Sep1992	11	3	53	4

5.2.2 Distribution surveys in agricultural grassland

Grassland surveys have been carried out for many years in the UK (eg Blackshaw, 1983a) but have never differentiated between *T. oleracea* and *T. paludosa.* A survey of agricultural grassland was carried out to determine the extent and distribution of *T. oleracea* in northern Britain.

Livestock farms were randomly selected from a database of all farms in Northern Ireland. Farm selection was stratified to reflect the farm size distribution within the Province. Once a farm had been selected it was left to the farmer to choose which field was to be sampled. A total of 75 farms was sampled between 8 January and 22 May 1991 (Table 5.2). Twenty cores of 10 cm diameter and 7.5 cm depth were taken in a "W" shape from each field. Cores were washed through a series of graded sieves under a high pressure hose. Larvae were floated off in saturated salt solution, washed in tap water and then frozen at -30°C until tested using IEF.

An annual leatherjacket survey has been carried out for over a decade in western Scotland. Site selection is non-random with more farms being surveyed in areas where either more grass is grown or an historic leatherjacket problem exists. Fields treated with insecticide are subsequently avoided in the survey for at least two successive years. Thus, on two occasions, different fields were surveyed on a farm over the two years covered by this study. Isoelectric focusing was used to identify leatherjackets from 67 of the fields surveyed between 30 October and 3 December 1990 and 25 January and 20 February 1991 (Table 5.3). Of the 129 fields surveyed between 29 October and 5 December 1991 testing was carried out on material from the 116 containing leatherjackets (Table 5.4). A further 117 fields were surveyed between 2 November and 9 December 1992 (Table 5.5).

For the survey twenty-five soil cores of 6.5 cm diameter and 6.5 cm depth were taken in a diagonal line from each field using plastic piping. Larvae were extracted in a modified Blasdale heat extractor (Blasdale, 1974) and then frozen until tested. Isoelectric focusing was carried out on a maximum of 10 larvae of representative size per field.

Farms in north-eastern Scotland are sampled in response to calls from farmers who are either in areas where leatherjacket problems occur frequently, or have spring cereals following grass. Thus, site selection is non-random and this accounts for the lack of any fields found to be without any leatherjackets. All material collected from the 14 fields sampled between 31 January and 5 March 1991 (Table 5.3) and the 18 sampled between 15 November 1991 and 24 January 1992 (Table 5.4) was tested. Soil sampling was as for western Scotland except that cores were taken in a "W" shape across each field.

Larvae were also obtained from an independent survey of 25 fields in January 1992 (Table 5.4) and 50 fields in January 1993 on Islay (Table 5.5). Sampling was as for western Scotland.

Since the food mixture used to rear leatherjackets did not affect the banding patterns of either species (Section 3.3), starvation of leatherjackets prior to testing was unnecessary. As a precaution, however, gut contents of field-collected material were initially removed from leatherjackets prior to maceration until it was ascertained that dietary material did not affect banding patterns.

Isoelectric focusing of larval protein was carried out on 723 leatherjackets from the 75 fields sampled in Northern Ireland. Thirty two samples produced banding patterns different from those of *T. paludosa* and *T. oleracea* and a further six produced no bands. Survey results, summarised in Table 5.2, showed that only one field contained *T. oleracea* (GR C661323) and this field did not seem to contain any other species.

In western Scotland IEF of 411 larvae recovered in 1990/91 revealed that 392 were *T. paludosa*, 18 were *T. oleracea* with one remaining unidentified. Four out of five occurrences of *T. oleracea* were on the Isle of Bute (GR NS385235, NS032671, NS037672, NS075663 and NS095571) (Table 5.3). A further 523 leatherjackets from western Scotland were tested by IEF in January 1992 (Table 5.4). Of these 514 were *T. paludosa*, five were *T. oleracea* (GR NR667192, NS053638, NS335442, NS452152 and NX724573), three were other species and one produced no bands. In 1992/1993 364 leatherjackets were tested (Table 5.5) of which 361 were *T. paludosa*. The remaining three larvae produced one other species, one with no bands and one indeterminate pattern (probably *T. paludosa*). No *T. oleracea* were found.

Table 5.2 ***Distribution by county of* T. paludosa *and* T. oleracea *in Northern Ireland between January and May 1991.***

County	Number of larvae		Number of fields surveyed		
	Tipula paludosa	*Tipula oleracea*	With *T.oleracea*	With no larvae	Total
Antrim	127	0	0	1	16
Armagh	27	0	0	0	6
Down	101	0	0	1	13
Fermanagh	184	0	0	0	12
Londonderry	77	3	1	2	12
Tyrone	166	0	0	2	16

Table 5.3 ***Distribution by county of* T. paludosa *and* T. oleracea *in western Scotland between October 1990 and February 1991 and in north-eastern Scotland between January and March 1991.***

County	Number of larvae		Number of fields surveyed		
	Tipula paludosa	*Tipula oleracea*	With *T.oleracea*	With no larvae	Total
Western Scotland					
Ayrshire	42	1	1	0	7
Argyll-Cowal	26	0	0	0	4
- Bute	98	17	4	0	15
Dumfries/Kirkcud	52	0	0	0	9
Lanarkshire	45	0	0	0	8
Renfrewshire	10	0	0	0	3
Stirling/Perth	12	0	0	1	7
Wigtownshire	107	0	0	0	15
North-eastern Scotland					
Aberdeenshire	17	0	0	0	6
Banffshire	19	0	0	0	4
Kincardineshire	3	0	0	0	1
Morayshire	19	0	0	0	3

Table 5.4 ***Distribution by county of* T. paludosa *and* T. oleracea *in western Scotland between October and December 1991, in north-eastern Scotland between December 1991 and February 1992 and in Islay in January 1992.***

County	Number of larvae		Number of fields surveyed		
	Tipula paludosa	*Tipula oleracea*	With *T.oleracea*	With no larvae	Total
Western Scotland					
Ayrshire	118	2	2	0	27
Argyll - Cowal	14	0	0	0	5
- Bute	73	1	1	1	15
- Kintyre	56	1	1	1	16
Dumfries/Kirkcud	78	1	1	3	20
Lanarkshire	83	0	0	1	14
Renfrewshire	18	0	0	0	6
Stirling/Perth	12	0	0	6	14
Wigtownshire	62	0	0	1	12
Islay	76	13	6	0	25
North-eastern Scotland					
Aberdeenshire	39	0	0	0	8
Banffshire	27	0	0	0	5
Invernessshire	10	0	0	0	2
Kincardineshire	6	0	0	0	1
Morayshire	19	1	1	0	2

Table 5.5 ***Distribution by county of* T. paludosa *and* T. oleracea *in western Scotland between November and December 1992 and in Islay in January 1993.***

County	Number of larvae		Number of fields surveyed		
	Tipula paludosa	*Tipula oleracea*	With *T.oleracea*	With no larvae	Total
Western Scotland					
Ayrshire	95	0	0	2	18
Argyll-Cowal-	21	0	0	0	5
Bute	72	0	0	1	15
Dumfries/Kirkcud	53	0	0	3	20
Lanarkshire	29	0	0	0	11
Renfrewshire	8	0	0	2	6
Stirling/Perth	19	0	0	6	14
Wigtownshire	34	0	0	3	12
Islay	162	12	7	13	50

All 58 larvae tested from the 1990/1991 survey in north-east Scotland produced banding patterns for *T. paludosa* (Table 5.3). In the 1991/1992 survey 107 larvae were tested of which 101 were identified as *T. paludosa* and one, from Morayshire, as *T. oleracea* (GR NJ394598). Three larvae produced no bands within pH 5-6 and a further two were assumed to be different species (Table 5.4).

The 1991/1992 survey of Islay resulted in 106 larvae, of which 76 were *T. paludosa*, 13 *T. oleracea*, 13 of other species, three with no bands and one indeterminate (probably *T. paludosa*) (Table 5.4). In 1992/1993, 174 leatherjackets were tested of which 162 were *T. paludosa* and 12 were *T. oleracea* (Table 5.5).

The leatherjacket surveys carried out in Northern Ireland and Scotland reveal that *T. oleracea* was very scarce in grassland in both regions. Overall, *T. oleracea* constituted 0.02% of the larvae tested compared with 96.7% for *T. paludosa*. The proportion of fields from which *T. oleracea* was recovered amounted to 5% of the total but the majority of these (18 out of 25 fields) were found in the Isles of Bute and, particularly, Islay. Reasons for this are, as yet, unclear although *T. oleracea* is thought to prefer wetter conditions (eg Brindle, 1960). The data presented here suggest that *T. oleracea* is not a major problem in grassland and so will not be the species responsible for damage to spring sown cereals that follow grass in a rotation. This is likely to be because dispersal of adult craneflies is part of normal

behaviour for this species (see Section 1) so that concentrations do not occur in stable habitats such as grassland.

5.2.3 Habitat preferences of larval T. oleracea

Information about the preferred larval habitat(s) of *T. oleracea* is very limited in the literature. In a habitat study carried out by den Hollander (1975) it was concluded that "*T. oleracea* males and females prefer high vegetation, composed of various plant species, and that *T. paludosa* males and young females prefer low vegetations, mainly composed of grasses."

Larval studies have been less conclusive. In northern England, Milne (1966) failed to ascertain the larval habitat of *T. oleracea* stating that "Unlike *paludosa, oleracea* does not spend its larval life in grassland here. Nor have we been able to find its larvae in neighbouring swampy patches, banks of streams etc. This is all very puzzling since the adults of both species can be equally plentiful in any light trap operating in the middle of any large area of grassland."

There are two likely explanations for this. One is the fact that the larval identification in the study must be treated with some caution because of the morphological similarity. Secondly, it is well documented that *T. oleracea* are better fliers and more active as adults and thus may have flown into the field. This study aimed first to locate adult *T. oleracea*, sampling a wide variety of habitats, and then to find the larvae in those habitats.

Ten sites in Northern Ireland were sampled between mid-May and the end of June 1993 using green water traps (Blackshaw, 1983b). Traps were serviced weekly and the presence/absence of *T. oleracea* adults recorded (Table 5.6). Larval sampling was carried out at sites where *T. oleracea* had been trapped. Twenty five 5 cm diameter soil cores were taken from all major secondary habitats within 50 m of traps recording adult *T. oleracea.* Larvae and pupae were extracted using a Blasdale heat extractor (Blasdale, 1984) and these were then identified using IEF. In addition, an area of approximately 6 ha at Flatfield, Co. Down, Northern Ireland (GR J194604) was systematically sampled after adult *T. oleracea* were observed flying during the summer of 1992. The area was divided into four, small, low-lying fields of which three were grazed, in rotation, by sheep. The fourth was cut for silage. Much of the land was prone to flooding from an adjoining river and the area provided a range of habitats; wet to dry soils, hedges, stream banks, rushes and pasture grasses.

Larvae were collected using the brine flotation technique, detailed elsewhere in this report (Section 4), between 13 and 16 November 1992. Pipes were sunk into the ground at 10 m intervals along the length of the field, with rows spaced at variable distances to include hedgerows, sides of ditches and other major field characteristics.

Results and Conclusions

Low numbers of adult *T. oleracea* were trapped at eight of the ten sites surveyed (Table 5.6). A single *T. oleracea* larva was recovered from grassland (used for silage production).

Table 5.6 ***Adult and larval distribution of* T. oleracea *in various habitats.***

Site	Water trap grid reference	Habitat type		Tipula oleracea	
		Primary	Secondary	Adults	Larvae
Aghalee	J113649	SCereals	Hedgerow	4	0
	J113648	SCereals	Hedgerow	4	0
	J111643	WCereals	Hedge, Ditch	1	0
Ballynahinch	J344519	GGrass	Hedgerow	0	-
	J3449506	GGrass	Hedgerow	0	-
Belfast	J333699	UGrass	Fallow	0	0
	J329698	C/UGrass	Poplars	1	0
	J331696	CGrass	Hedgerow	0	0
Crossgar	J447514	UGrass	Hedgerow	0	0
	J446513	UGrass	Streambank	2	0
	J443515	DWoods	Streambank	0	0
Flatfield	J195605	GGrass	Hedgerow	1	0
	J194604	GGrass	Hedgerow	0	0
Greenmount	J175842	Hedgerow	CGrass, Marsh	0	-
Hillsborough	J245576	CGrass	Hedge, DWood	1	1
	J45575	DWood	Hedge, Grass	0	0
	J248573	CWood	-	0	0
Oxford Island	J046622	AWood	Grass	1	0
	J047617	Marsh	-	0	0
Saintfield	J372573	GGrass	Hedge, Ditch	3	0
Stoneyford	J215705	GGrass	-	2	0

S=spring, W=winter, G=grazed, C=cut, U=uncut and ungrazed,
D=mixed deciduous, C=coniferous, A=Alder.

The systematic sampling of the grassland area at Flatfield yielded 18 leatherjackets (Table 5.7). Seven were in close proximity to hedgerows, three at the sides of ditches and eight within the main field areas but all were *T. paludosa.*

Table 5.7 Systematic leatherjacket survey of a 6.15 ha grassland area.

				Leatherjackets	
Field	Field size (ha)	No. of rows	Total no. of pipes	*oleracea*	*paludosa*
1	1.22	6	42	0	6
2	1.79	5	87	0	4
3	1.55	5	95	0	5
4*	1.59	3	59	0	3

* Partly flooded

Despite the fact that adult *T. oleracea* were observed and trapped over agricultural land in this study, there was no substantive evidence that larvae are found in the same habitats. This reinforces the observation of Milne (1966) concerning larval distribution and suggests that adults are both mobile and selective about oviposition sites.

6. INFLUENCE OF OILSEED RAPE ON *TIPULA OLERACEA* DYNAMICS

6.1 Movement within oilseed rape

6.1.1 Influence of the canopy on dispersal

If *T. oleracea* larvae overwinter in an oilseed rape crop then adults can be expected to emerge in May or June. At this stage of the growing season, individual plants are large and the canopy is complex and interlocking.

Adult *T. oleracea* have a winglength of 18-28 mm (Coe *et al*, 1950) and therefore a wingspan of up to 6 cm. Legs are also long. The size and structure of the cranefly may prevent it from penetrating the canopy of oilseed rape.

Two cages were constructed from 2 cm wooden battens. The traps measured 210 cm x 75 cm to allow for clearance of an oilseed rape crop which can reach 1.8m in height. The sides of the cages were covered with 500 gauge black polythene sheeting to eliminate all light sources. The sheeting was glued onto the frame and was further secured by nailing. A perspex square, 6 mm x 75 cm x 75 cm was secured on to the top of the frame using 8 bolts (5 mm x 42 mm) and wing nuts. The underside of the perspex, ie the side facing into the crop, was covered with a non drying adhesive (Boltac Grease). The cages were staked into position in the field with guy ropes attached to each corner. An extra lip of sheeting was attached to the bottom of the cage and was stapled directly into the ground to prevent any of the trapped insects from crawling out.

The traps were placed in adjoining fields of grass and oilseed rape. The sites were located at Muchalls Farm, Newtonhill, Aberdeenshire, Grid Ref No 875925 in 1990 and at Tillycorthie Farm, Udny, Aberdeenshire, Grid Ref NJ 911234 in 1991. The oilseed rape variety was Samourai in 1990 and Cobra in 1991. In both years the grass trap was placed on a sward consisting of ryegrass, timothy and white clover.

Equal numbers of adults, in the same sex ratio, were taken from a laboratory culture and released into each trap on each date. The craneflies were attracted to the light at the top of the cage and were trapped by the adhesive. The perspex was detached for inspection and trapped flies removed. In 1990 the traps were checked 2 and 4 days after release and the number of adult flies trapped on the perspex sheet recorded. The experiment was repeated three times during 1990. After each experiment the cages were moved to another part of the field. In 1991 the traps were checked only once, 4 days after the release date. The experiment was repeated on 12 occasions.

Chi-squared tests were applied to the data using the total number of flies recaptured in the grass trap as "expected" and the total number of flies recaptured in the oilseed rape trap as "observed".

In 1990 there were significantly (X^2=13.17; $P < 0.05$) fewer craneflies recovered from the trap over the oilseed rape than that over the grass (Table 6.1). A similar result was obtained in 1991 (Table 6.2) with fewer females (X^2=25.53; $P < 0.01$), males (X^2=64.75; $P < 0.001$) and total craneflies (X^2=91.62; $P < 0.001$) being recovered from the rape. These two studies demonstrate that the rape canopy can impede the normal dispersal behaviour of *T. oleracea.*

In 1991, however, there were no significant differences between total numbers recovered for the first two sampling dates (X^2=3.34) or male recaptures for the first three sampling dates (X^2=3.74). Thus the rape needs to reach a growth stage of approximately 4.3 (30% of buds open) before the canopy seriously affects the cranefly's ability to disperse.

In the 1991 study (Table 6.2) the ratio of males recovered from rape to males recovered from grass (0.227) was greater than that for females (0.068) which indicates a differential effect on the two sexes. This would be expected because male *T. oleracea* tend to be smaller and more active fliers.

Table 6.1 ***Recovery of adult* T. oleracea *from emergence traps over grass and oilseed rape in 1990.***

		Numbers recovered after:			
		2 days		4 days	
Release date	Numbers released	Grass	OSR	Grass	OSR
8 May	11	10	6	0	1
16 June	8	7	2	0	0
24 June	10	8	0	1	0
Total	29	25	8	1	1

Table 6.2 ***Recovery of female (f) and male (m) adult T. oleracea from emergence traps over grass and oilseed rape in 1991.***

Release date	OSR growth stage	Numbers released		Number recaptured		Grass	OSR
		f	m	f	m	f	m
25 April	3.6	8	10	3	7	1	6
9 May	4.0	9	9	2	5	0	2
16 May	4.3	7	8	4	5	0	2
24 May	5.3	8	12	2	7	0	0
7 June	5.5	9	8	2	4	0	1
21 June	5.9	8	6	2	5	0	1
26 June	6.2	8	10	1	9	0	3
5 July	6.2	9	16	6	12	1	2
15 July	6.3	8	12	5	8	0	1
24 July	6.4	6	12	0	10	0	2
8 August	6.7	16	18	0	15	0	2
16 August	6.9	8	15	2	14	0	1
Total		104	136	29	101	2	23

6.1.2 Lateral movement by craneflies beneath the rape canopy

The rape canopy impedes vertical movement by *T. oleracea* and so adults must seek for a mate within the crop. To be successful, they must be able to move laterally away from their pupation sites.

At crop establishment, winter rape plants may be quite closely packed - 100 plants or more m^{-2}. However the plant density declines throughout the growing season to approximately 80 plants m-2 in the spring and perhaps about 70 plants m^{-2} at harvest time (K. Walker, personal communication). In addition, the upper foliage of the oilseed rape plants become so densely packed and intertwined that sunlight does not reach the lower part of the stalks. Consequently the lower part of the stalks become devoid of vegetation. In effect these two factors allow the development of open space beneath the canopy of the oilseed rape.

A preliminary experiment was undertaken in the laboratory to test the experimental approach. A square sided tunnel was made from black polythene sheeting. The tunnel was

approximately 50 cm wide. A clear piece of perspex coated with a non-drying adhesive was attached to one end of the tunnel. A flap, through which craneflies were introduced, was cut at the other end of the tunnel. A 100 w light bulb was used to shine light through the perspex end of the tunnel. The experiment was carried out in a dark room. Craneflies attracted to the light source became trapped on the glue and the total number caught were recorded after three days.

Three different lengths of tunnel were examined (1, 2 and 3 m). Flies were released into each length of tunnel on 10 occasions; five releases were of females and the other five of males. Different numbers of flies were released on each date depending upon their availability from cultures.

The second experiment took place in a field at Tillycorthie Farm, Udny in August 1993. Oilseed rape stubble was present in the field which had recently been harvested. Black plastic sheeting was draped over the rape stubble at a height of approximately 50 cm. A similar tunnel was constructed over areas where the stubble had been cut and removed ("cut rape"). Short canes were inserted at the corners to stabilise the tunnel. The edging of the tunnel was doubled over and was secured to the ground using tent pegs. One end of the tunnel could be opened and this facilitated the release of craneflies. A perspex sheet covered in non-adhesive glue was secured at the other end of the tunnel. Flies were released into the tunnel and the number caught on the glue recorded after 4 days. Tunnel lengths of one, two and three m were tested. Flies were released into both traps on six occasions at each length; three releases were all females and three were males.

Results and Conclusions

There was no significant difference in the proportions of released female (67%) and male (64%) craneflies recovered in the laboratory study. Recaptures did, however, decrease with distance away from the light source (Table 6.3) and there was a significant difference ($P<0.05$) for those released at distances of one and three m.

Table 6.3 ***Movement of craneflies towards a light source in the laboratory (Sums of five releases).***

Distance (m) from light	Females		Males	
	Released	% caught	Released	% caught
1	48	77	56	75
2	37	67	47	70
3	38	52	53	47

Results from the field study in cut rape were similar with 49% of females and 48% of males recaptured after release. Recaptures also decreased with distance from the light source (Table 6.4) and, again, there was a significant difference ($P < 0.05$) in recovery rates between 1 and 3 m.

Table 6.4 ***Movement of craneflies towards light in cut rape (Sums of three releases).***

Distance (m)	Females		Males	
from light	Released	% caught	Released	% caught
1	16	63	12	58
2	13	61	18	55
3	24	33	18	33

With the trap placed over the rape stubble (Table 6.5), 32% of released females were recovered in comparison with 41% of males but this difference was not statistically significant. Although most craneflies were recovered when released 1 m from the light, there was no significant effect of release distance on numbers subsequently caught.

Overall, a greater proportion of released craneflies were recovered from the trap set over the cut rape (49%) than from that over rape stubble (36%). This difference was not significant.

Table 6.5 ***Movement of craneflies towards light in rape stubble (Sums of three releases).***

Distance (m)	Females		Males	
from light	Released	% caught	Released	% caught
1	16	44	5	50
2	16	25	10	40
3	24	29	14	36

These experiments have demonstrated that male and female *T. oleracea* are both equally capable of moving towards light and that this movement is not significantly impeded by the stems of rape plants. It is therefore probable that other behavioural responses (eg mating and oviposition) will not be limited within the lower horizons of a rape crop and that the life-cycle can be completed in this environment.

6.2 Effect on larval survival and growth

6.2.1 Survival of T. oleracea *on diets of rape and wheat*

For larvae of *T. oleracea* to survive and grow within an oilseed rape crop they must be able to find food. When the rape is establishing it is common to find a range of weed species associated with the crop. Chickweed, annual meadow grass, volunteer cereals, shepherd's purse, fumitory and pansy are the weed species most commonly found in oilseed rape crops in north-east Scotland (Whytock and Carnegie, 1990). However, as the oilseed rape crop establishes itself it usually smothers the weed flora so that, during the summer months, there are very few weeds growing underneath the dense canopy of the oilseed rape. At this time of year there are few available alternatives to rape, or its products, as a food source for the leatherjackets.

Twenty 17.8 cm diameter pots were filled with 3 mm, sterilised sharp sand. Ten, three-week old, laboratory reared *T. oleracea* larvae were added to each pot. The larvae were derived from two sets of parents but equal numbers from each were divided between the two treatments.

Seedlings of winter wheat (cv. Riband) and oilseed rape (cv. Rocket) were grown and seedlings harvested as feed material two days after germination. Larvae in ten of the pots were fed with wheat and the ten remaining pots were fed with a diet of oilseed rape. The plant material was provided *ad libitum* to each of the pots. The pots were arranged alternately in a greenhouse to avoid any site bias. The larvae were weighed individually at the beginning of the experiment. After three and six weeks the larvae were removed by hand from the pots and the number of survivors recorded.

Results and Conclusions

Twenty percent of the larvae fed on oilseed rape diets died in the first test period i.e. 0-3 weeks (Table 6.6). Of the survivors, 21% died in weeks 3-6, so that in total 37% of larvae fed on oilseed rape died in the six week trial period. This compares with the 35% of larvae fed winter wheat which died in the first three weeks of the trial period and the 29% of survivors that died in weeks 3-7. Over the six week period 54% of the larvae fed on the diet of winter wheat died.

More larvae survived for six weeks when fed on a diet of oilseed rape compared to those fed on a diet of winter wheat (X^2=5.84; $P < 0.05$). A significant difference (X^2=5.66; $P < 0.05$) between the two diets was also recorded in the first three weeks of the trial and this suggests that the bulk of the differential in mortalities occurred during this period.

Table 6.6 ***Survival of* T. oleracea *on diets of oilseed rape and winter wheat (n=100).***

	Percentage survival	
	3 wks	6 wks
Oilseed rape	80	65
Winter wheat	63	46

6.2.2 Growth of T. oleracea *on diets of rape and wheat.*

In addition to being able to survive on a diet of oilseed rape foliage, leatherjackets also need to be able to grow in order to complete development.

Three age groups of 2 (young), 4 (medium) and 8 (mature) weeks old larvae were used in the experiment arising from hatchings on the 28 June, 13 June and 24 May respectively. Fifty larvae were randomly selected from each age group. Twenty five of these larvae were fed on oilseed rape and twenty five were fed on winter wheat.

The larvae were kept separately in open-ended tubes with ventilated removable caps at each end. This allowed access for feeding and also allowed water drainage. The tubes were filled with moistened, sterilised, 3 mm, sharp sand and arranged alternately, by diet, in an incubator (16°C). The larvae were fed with fresh plant material grown in the greenhouse. Seedlings of oilseed rape and wheat were used as feed at 2 days post-emergence.

Larvae were weighed individually at the start of the experiment and at weekly intervals thereafter.

Results and Conclusions

Growth rates were calculated using the final weight of larvae which survived the whole experimental period. In all of the age groups tested fatalities occurred and some individuals reached pupation. Data for these larvae were omitted from the growth rate calculation because they showed anomalous behaviour before death and pupation i.e. they lost weight in the preceding weeks.

The 'mature' larvae group showed a weight loss in the first week of the experiment on both the oilseed rape and wheat diets (Fig 6.1). After this period the larvae grew. This weight loss was not detected in the two other age groups, young and medium, on either of the diets (Figs 6.2 and 6.3).

The results from the comparison of the mean growth rates (Table 6.7) show that the 'mature' larvae group grew significantly ($P < 0.05$) better on the oilseed rape diets than on the winter wheat diets. The final weight of both 'mature' sets of larvae was similar but the group fed on the oilseed rape started at a lower initial weight (Fig 6.1).

Fig 6.1 Growth of 'mature' larvae on different diets

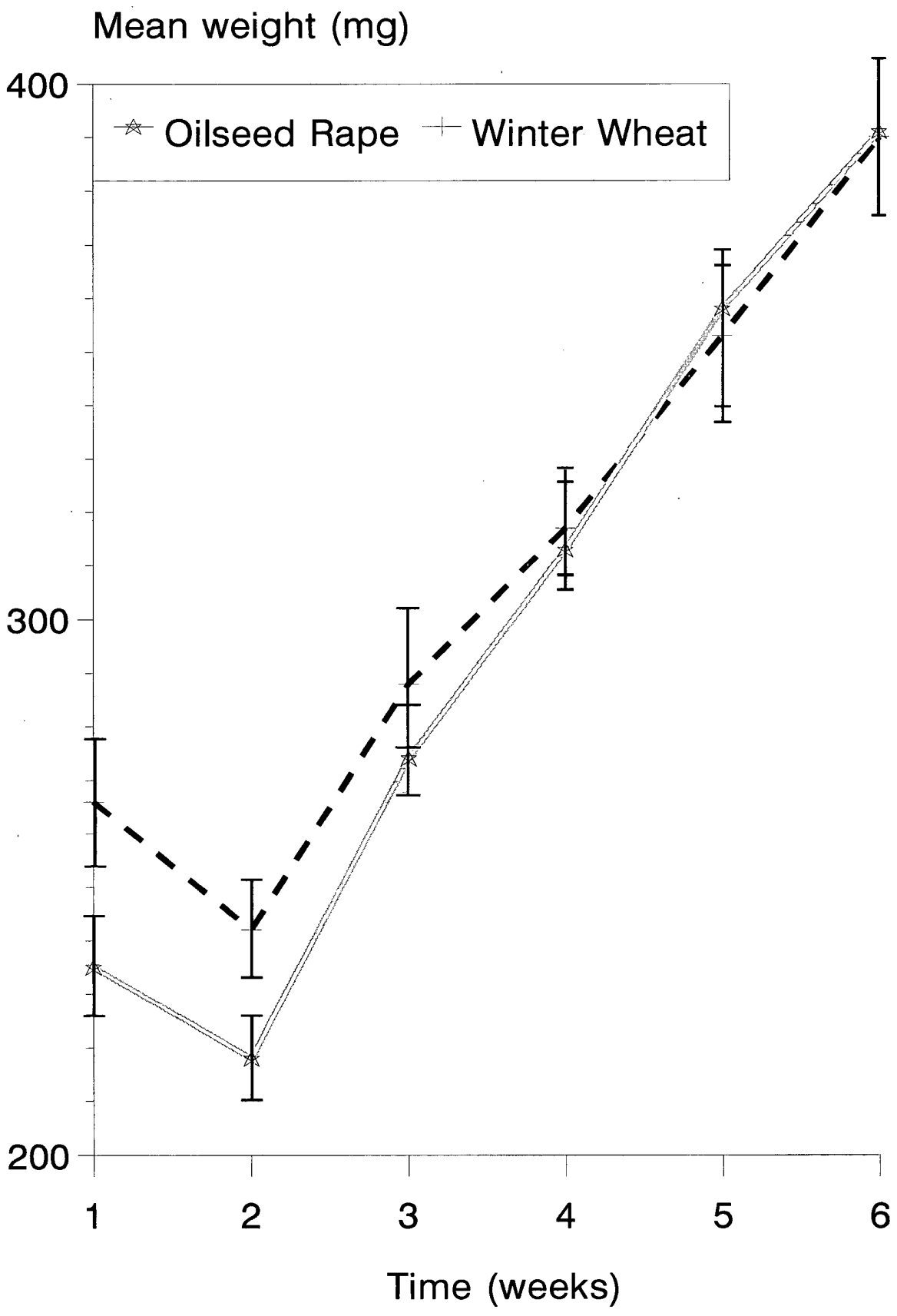


Fig 6.2 Growth of 'medium' larvae on different diets

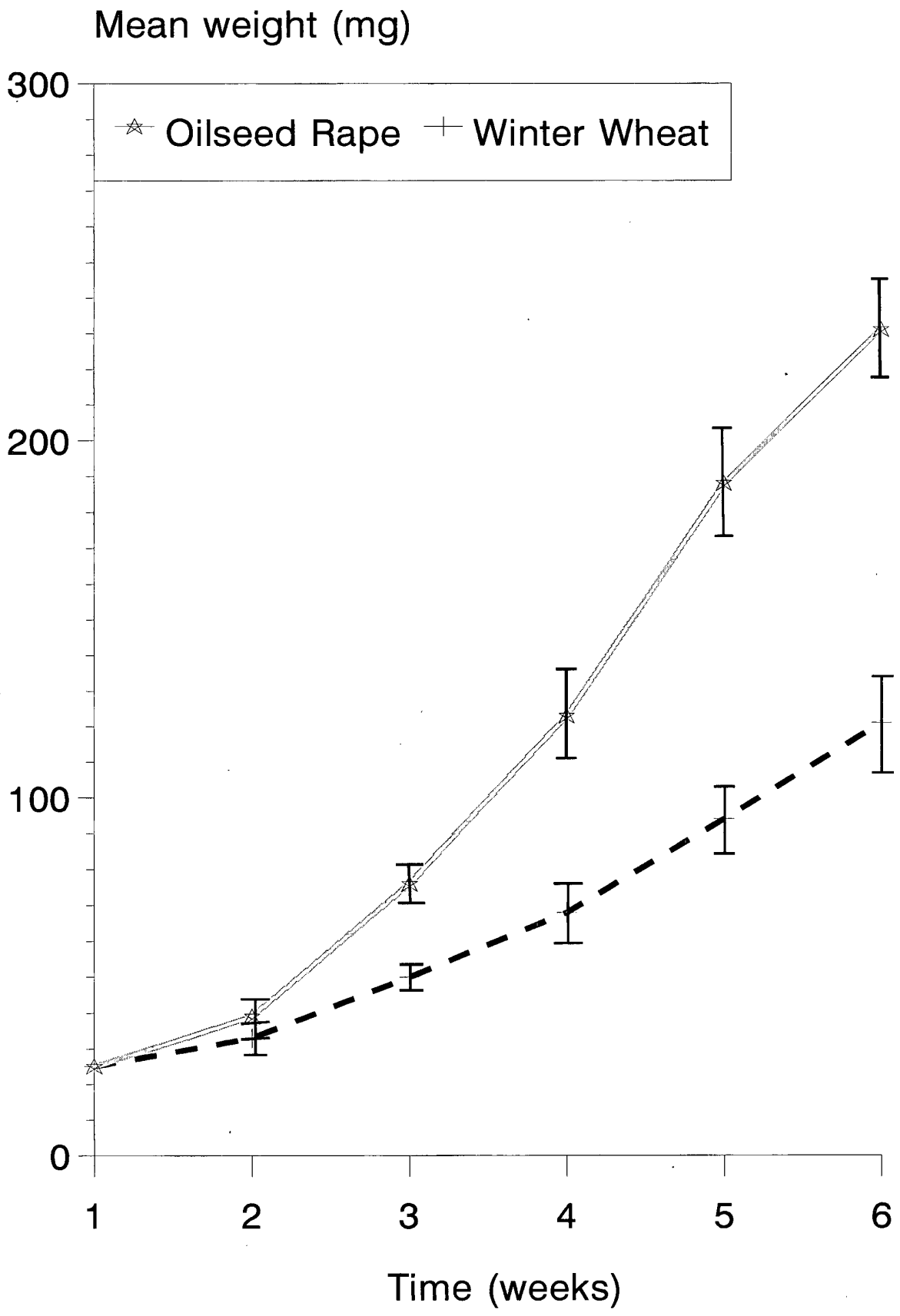
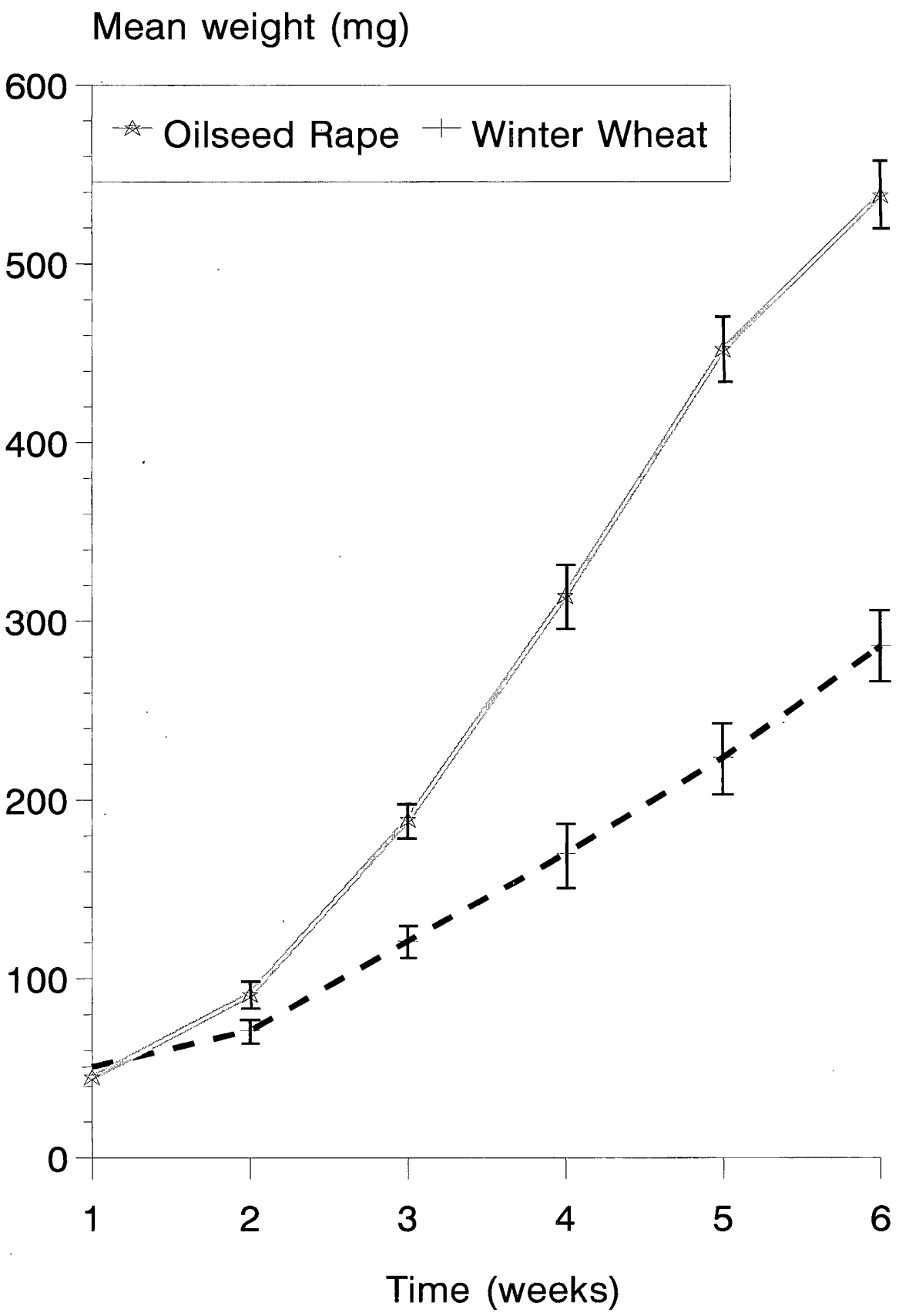


Fig 6.3 Growth of 'small' larvae on different diets



The larvae from the 'young' and 'middle' age groups started the experimental period at similar mean weights. However in both of these age groups the larvae fed on diets of oilseed rape achieved a significantly ($P < 0.05$) greater final weight. A comparison of the mean weights (Table 6.7) shows that larvae in both the 'young' and 'medium' age groups had significantly higher growth rates than the larvae fed on wheat.

Table 6.7 ***Influence of diet on mean growth rate (mg wk-1) of*** **T. oleracea** ***larvae.***

	Diet	
Age at start (wk)	Wheat	Oilseed rape
8	31.99	41.32
4	23.09	51.41
2	29.47	61.95

The results show that the larvae from each age group survived and continued their development when fed on diets of both winter wheat and oilseed rape, with some pupating and emerging as fully formed adult flies. Rape provides an adequate food source to support *T. oleracea* growth and may represent a preferred habitat because the rate of weight increase on this diet was considerably greater than that achieved with winter wheat.

7. DISCUSSION

Initial farmer and adviser reaction to the discovery of leatherjackets in winter cereals assumed that this was a manifestation similar to that experienced with *T. paludosa* in spring barley. This project has established that damage to winter cereals in northern Britain is primarily caused by *T. oleracea*. Its genesis as a pest is not because it has changed its biology but because agricultural conditions have altered.

Many species of invertebrates have reached pest status because they have successfully adapted to specific favourable conditions provided by agricultural systems used in the UK. The female wheat bulb fly, *Delia coarctata,* lays her eggs in bare soil (Anon, 1985) and this species has proved to be a nuisance in winter cereal crops which follow a fallow in the rotation (Anon, 1986). Pollen beetles, *Meligethes* spp., have escalated in numbers in oilseed rape crops particularly since 1987 (Walters and Lane, 1992). Large populations of the cabbage rootfly, *Delia radicum,* have built up in areas where brassicas are grown intensely (Jones and Jones, 1984) whereas its close relative the turnip root fly, *Delia floralis,* has found favour with crops of swedes and turnips grown in northern areas only (McKinlay, 1992). As a group, insects are successful crop pests because they are efficient opportunists and also because they have many physical advantages which allow them to adapt to changing conditions. They are mobile, often have high reproduction rates and they possess a variety of strategies for over wintering and camouflaging themselves from predators. Therefore it comes as no suprise that the advent of a new cropping system, the introduction of a new crop or any change in land use will often herald a new pest scenario.

In the case of *T. oleracea* the important change has been the introduction of oilseed rape into arable rotations. In Scotland the area of oilseed rape grown has risen from 120 ha in 1981 to 71,000 ha in 1994 (Table 7.1). Oilseed rape with its mass of yellow flowers and large quantities of pollen and nectar is a very lucrative crop to many species of insects, both pests and beneficials (Anon, 1991). Seed weevils, flea beetle, aphids, midges, cabbage root fly and honey bees are all easily found in oilseed rape crops (Paul and Rawlinson, 1992). With such a high insect association, a higher pest incidence might be anticipated. This has already been noted in the increased risk of slug damage in crops following oilseed rape in the rotation (Stephenson and Bardner, 1976).

Table 7.1 Growth of oilseed rape in UK and Scotland

Harvest Year	UK ha	Scotland ha
1977	55,000	not available
1981	105,000	120
1984	269,000	11,050
1987	388,000	45,000
1989	321,000	36,000
1990	391,000	45,190
1991	440,000	49,890
1992	421,000	56,860
1993	417,613	59,930
1994	493,810	70,910

(Anon, 1994b)

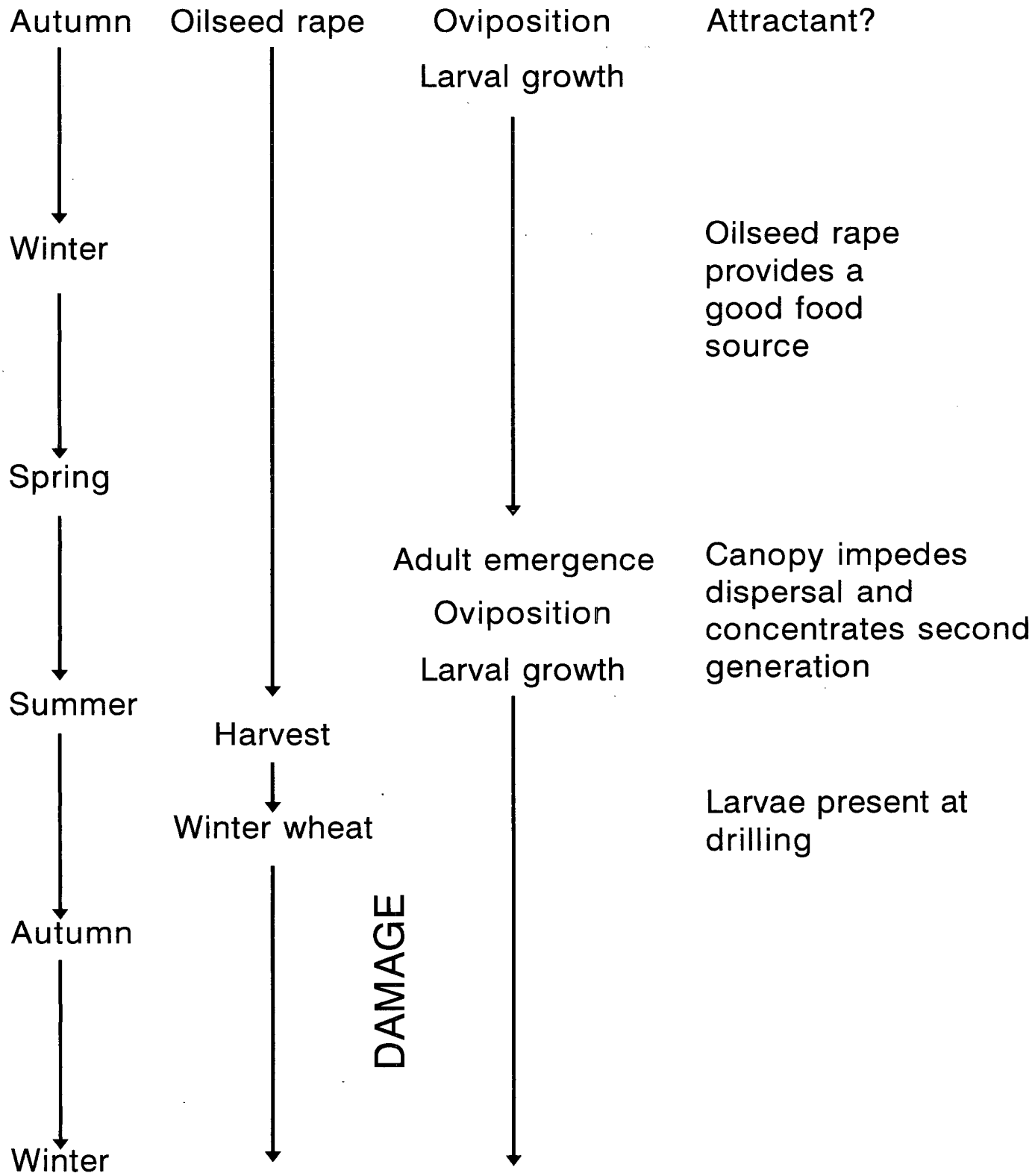
Greater numbers of *T. oleracea* may be found in rape crops compared with wheat in the autumn and at a higher frequency (Table 5.1). Numbers increase in the rape crop during the growing season (Table 5.1), mainly because adult dispersal is impeded by the canopy (Section 6.1). Larval survival and growth is also better in rape than in wheat (Section 6.2) and this suggests that it is a preferred host.

This project failed to discover the habitat(s) in which larvae of *T. oleracea* are normally to be found (Section 5.2) and reached similar conclusions to those of Milne (1966). The exception to this was the concentration in Bute and Islay. These few findings, however, are of little relevence to the major cereal growing areas of Scotland and Northern Ireland and the distribution of *T. oleracea* larvae in these areas remains unknown. It is possible that populations in agricultural land, especially grassland, are widely dispersed so that individual larvae are infrequently encountered.

That larvae are found in oilseed rape relatively easily within these areas suggests that adults are being attracted into the crop to oviposit. This is a gap in our current knowledge and warrants further study.

Our current understanding of the epidemiology of *T. oleracea* damage to winter cereals can be summarised (Fig 7.1). A number of critical points of control opportunity occur. The first is at oviposition in rape in the autumn. If there is an attractant operating its discovery may enable the development of preventative strategies that would reduce the frequency of damage. The second control opportunity is during the early stages of crop growth, before the canopy closes over, when larvae may be accessible to insecticides. Unfortunately, none of the sampling methods studied in this project (Section 4) would be effective in monitoring larval numbers at this stage.

Fig 7.1 Damage cycle for Tipula oleracea



Once the canopy has closed over both sampling and control become unrealistic. The next opportunity is after harvest when stubble can be sampled. Here, the brine flotation method can be used and will provide reliable estimates of leatherjacket population levels (Section 4).

Finally, row scratching can be applied in the winter cereal crop after emergence. The problem with this approach is that numbers of leatherjackets in winter wheat can be so high (>5 m ha^{-1}) that the crop is rapidly destroyed before monitoring begins. Experience with leatherjackets in spring barley suggests that damage from much smaller populations can be significant before remedial applications are applied (Blackshaw *et al.*, 1994).

Some farmers in north-east Scotland have encountered problems with chemical control of leatherjackets in late autumn/early winter in wheat with apparent failure of insecticides such as chlorpyrifos (C. Coll, unpublished data). Repeat applications have been necessary. At present there is no reason to suppose that efficacy against *T. oleracea* is significantly less than that against *T. paludosa*. A more plausible explanation of these observations is that control is incomplete. Rayner (1975) achieved a 72% mortality of leatherjackets in spring barley with chlorpyrifos, suggesting that a significant proportion of any population may survive insecticide application. With large numbers present, say 5 m ha^{-1}, even a 90% kill would leave a residual population of 0.5 m ha^{-1}, sufficient to cause considerable damage to a winter cereal crop.

Remedial action in the growing cereal crop is too late. The optimal time for farmers to interupt the *T. oleracea* damage cycle is after harvest of oilseed rape. At this point leatherjacket populations can be assessed in the stubble and chemical control implemented to prevent loss to the succeeding cereal crop. Although brine flotation is the recommended sampling method it should be recognised that the time and physical resources required to operate it have detracted from its adoption (Blackshaw, 1994). The bait board method could provide an indication of the need to sample through the detection of leatherjackets in rape stubbles. Before this could be recommended, however, we would need to know the environmental thresholds for leatherjacket activity and this requires further work.

In addition to a preceding crop of oilseed rape, the risk of T. oleracea attack in any field will be increased by a short harvest interval and wetter soils. Damage may, however, be reduced by rolling and farmers should be encouraged to include this as part of normal husbandry of winter cereals.

It should be noted that there is some evidence that other crop rotations may also give rise to similar problems. In Suffolk, damaging levels of leatherjackets were found in winter wheat that had followed field beans (R P Blackshaw, unpublished data) and in Northern Ireland a spring barley crop in 1986 that followed winter oilseed rape in 1985 and spring barley in 1984 had, unusually, a population above the economic threshold (Blackshaw, 1988). Both of these examples involved previous crops with extensive and complex canopies that might be expected to impede *T. oleracea* dispersal.

Determination of leatherjacket population levels prior to seed-bed preparation for winter cereals is only part of the answer. The relationship between population levels and yield loss has not yet been established and the efficacy of insecticides in oilseed rape stubbles needs to be estimated. Without these data economic thresholds cannot be calculated; future research needs to address these topics in order to complete the work started in this project.

In the absence of data, advice to farmers must remain tentative. The threshold for leatherjacket control in spring barley is generally accepted to be 0.6 m ha^{-1} (Anon, 1994), although historical data indicates a lower value (Blackshaw *et al.*, 1994). Spring crops are likely to be able to better grow away from feeding damage. Conservatively, this may indicate a threshold in winter cereals of about half the spring level, or 0.3 m ha-1. Taking into account a 70% mortality due to seed-bed preparation (Blackshaw, 1988), the economic threshold in the oilseed rape stubble may be around 0.43 m ha^{-1}, or an average 0.34 leatherjackets per pipe using the brine flotation method.

This project has identified causal factors for the occurrence of leatherjacket damage in winter cereals in northern Britain. The principal feature is a preceding crop of winter oilseed rape and farmers in affected regions should anticipate problems. They are recommended to (a) monitor rape stubbles for leatherjackets, (b) apply insecticides if necesary and (c) routinely roll after drilling.

8. REFERENCES

Ahmed, M. (1968) *A study of the Tipulidae (Diptera) of agricultural importance in south-east Scotland.* Ph.D. Thesis University of Edinburgh

Alford, D.V. (1984) *A Colour Atlas of Fruit Pests; their biology recognition and control.* Wolfe Publishing Ltd.

Anon (1953) Leatherjackets, 1951-1952. *Plant Pathology* 2, 136

Anon (1981) Leatherjackets and their control. *The Scottish Agricultural Colleges Publication No. 77*

Anon (1984) Leatherjackets. *ADAS Leaflet 179, Ministry of Agriculture Fisheries and Food.*

Anon (1985) Wheat bulb fly. *ADAS leaflet 177, Ministry of Agriculture Fisheries and Food.*

Anon (1986a) Chemical control and ecological regulation of the cranefly (*Tipula paludosa* Meigen; Diptera, Insecta) on farmland in North-west Germany. *Nature Special Report. Number 2.* Wardenburg.

Anon (1986b) Wheat bulb fly. *Scottish Agricultural Colleges, Publication No. 169*

Anon (1989) Potato Pests. *ADAS Reference Book 187, London.*

Anon (1991) Insect pests of oilseed rape in Scotland, *SAC Technical note T284* Cleeve Gardens Oakbank Road, Perth PH1 1HF

Anon (1994a) Leatherjackets and their control, *SAC Technical note T361* Cleeve Gardens Oakbank Road, Perth PH1 1HF

Anon (1994b) *Agricultural Statistics Census (1989-1994).* Scottish Agricultural Census, Department of Agriculture for Scotland, Edinburgh.

Aveling, C. (1981) Action of mephosfolan on anthocorid predators of *Phorodon humuli. Annals of Applied Biology* 97, 155-164

Barbash, N.M., Coll, C. and Dobson, R.M. (1991) Observations on the overwinter mortality of the larvae of *Tipula paludosa* Meigen (Diptera: Tipulidae) due to bird predation. *The Scottish Naturalist*, 73-84

Barnes, H.F. (1936) Methods of investigating the bionomics of the common cranefly, *Tipula paludosa* Meigen, together with some results. *Annals of Applied Biology* 14, 356-368.

Beintema, A.J., Thissen, J.B., Tensen, D. and Visser, G.H. (1991) Feeding ecology of charadriiform chicks in agricultural grasslands. *Ardea* 79, 31-44

Blackshaw, R.P. (1983a) Some factors influencing variability in water traps of *Tipula* spp. (Diptera: Tipulidae). *Bulletin of Entomological Research* 73, 693-699

Blackshaw, R.P. (1983b) The annual leatherjacket survey in Northern Ireland, 1965-82, and some factors affecting populations. *Plant Pathology* 32, 345-349

Blackshaw R.P. (1984). The impact of low numbers of leatherjackets on grass yield. *Grass and Forage Science* 39, 339 -344

Blackshaw, R.P. (1985) A preliminary comparison of some management options for reducing grass losses caused by leatherjackets in Northern Ireland. *Annals of Applied Biology* 107, 279-285

Blackshaw, R.P. (1988) Effects of cultivations and previous cropping on leatherjacket populations in spring barley. *Research and Development in Agriculture* 5, 35-37

Blackshaw, R.P. (1990) Observations on the distribution of leatherjackets in Northern Ireland. *Annals of Applied Biology* 116, 21-26

Blackshaw, R.P. (1991) Leatherjackets in Grassland. *Proceedings of the Bristish Grassland Symposium on Strategies for Weed, Disease and Pest Control in Grassland*, pp. 6.1-6.10

Blackshaw, R.P. (1994) Sampling for leatherjackets in Grassland. *Aspects of Applied Biology* 37, 95-102

Blackshaw, R.P. and Newbold, J.W. (1987) Interactions between fertilizer use and leatherjacket control in grassland. *Grass and Forage Science* 42, 343-346

Blackshaw, R.P. and Perry, J.N. (1994) Predicting leatherjacket population frequencies in Northern Ireland. *Annals of Applied Biology* 124, 213-219

Blackshaw, R.P., Stewart, R. M., Humphreys, I. C. and Coll, C. (1994) Preventing leatherjacket damage to cereals. *Aspects of Applied Biology* 37, 189-196

Blasdale, P. (1974) A method of turf sampling and extraction of leatherjackets. *Plant Pathology* 23, 14-17

Bodenheimer, F. (1923) Beitrage zur Kenntnis von *Tipula oleracea* L. *Zeitschrift fur Angewandte Entomologie* 9, 1924

Brindle, A. (1959) Notes on the larvae of British Tipulinae (Dipt., Tipulidae). part 6, The larvae of the *Tipula oleracea* group. *Entomologist's Monthly Magazine* 95, 176-177

Brindle A. (1960) The larvae and pupae of the British Tipulinae. *Transactions of the Society for British Entomology* 14(3), 63-114

Brownbridge, M. and Selman, B.J. (1989) Improvements in the laboratory rearing of cultures of leatherjackets (*Tipula* spp.) (Dipt., Tipulidae) *Entomologist's Monthly Magazine* 125, 123-127

Cameron, A. E. (1917) The insect association of a local environment complex in the district of Holmes Chapel, Cheshire. *Transcripts of the Royal Society of Edinburgh* 52, 54-78.

Campbell R. L. (1975) Insecticidal control of European Crane fly in Washington. *Journal of Economic Entomology* 68, 386-391

Carter, J.B. (1973) The mode of transmission of Tipula Iridescent Virus. I Source of infection. *Journal of Invertebrate Pathology* 21, 123-130

Carter, J.B. (1976) A survey of microbial, insect and nematode parasites of Tipulidae (Diptera) larvae in north-east England. *Journal of Applied Ecology* 13, 103-122

Carter, J.B. (1978) *Tipula iridescent* virus infection in the developmental stages of *Tipula oleracea*. *Journal of Invertebrate Pathology*. 24, 271-281

Carter, J.B., Griffiths,C. and Smith., A.D. (1981) A high level of parasitism by *Siphona geniculata* (De Geer) (Diptera: Tachinidae) in a population of *Tipula paludosa* (Meigen) (Dipt. Tipulidae). *Entomologist's Gazette* 32, 257-258.

Chapman, P (1994). Control of leatherjackets by natural enemies: the potential role of the ground beetle, *Pterostichus melanarius*. *Proceedings of the British Insecticide and Fungicide Conference* pp. 933-934

Chiswell, J. R. (1956) A taxonomic account of the last instar larvae of some British Tipulinae (Diptera: Tipulidae). *Transactions of the Royal Entomological Society of London* 108, 409-484

Clements, R.O. and Bale J.S. (1988) The short term effects on birds and mammals of the use of chlorpyrifos to control leatherjackets in grassland. *Annals of Applied Biology* 112, 41-47

Clements, R.O., Murray, P.J. and Tyas, C.J. (1992) The short - term effects on wild goose behaviour of chlorpyrifos application to permanent pasture. *Annals of Applied Biology* 120, 17-23

Coe, R.L., Freeman, P. and Mattingly, P.F. (1950) *Diptera: Nematocera. Handbooks for the Identification of British Insects 9, Part 2.*

Cohen, M. (1953) Survey of leatherjacket populations in England and Wales 1946-51. *Plant Pathology* 2, 80-82

Coyler, C.N. and Hammond, C.O. (1951) *Flies of the British Isles.* Warne & Co. Ltd. London, New York.

Coulson, J.C. (1959) Observations on the Tipulidae (Diptera) of the Moor House Nature Reserve, Westermorland. *Transactions Royal Entomology Society of London* 111, 157-174

Coulson, J.C. (1962) The biology of *Tipula subnodicornis* Zetterstedt wth comparative observations on *Tipula paludosa* Meigen. *Journal of Animal Ecology* 31, 1-21

Cuthbertson, A.. (1929) The mating habits and oviposition of craneflies. *Entomologist's Monthly Magazine* 65, 141-145

De Jong, W.H. (1925) Een studie over emelten en hare bestrijding. *Verslagen en Meddeedelingen Plantenziektenkundige Dienst Waageningen* 42, 1-126

Del Guercio, G. (1914) Le Tipuli ed i Tafani nocivi nelle Risaie di Molinell (Bologna). *Redia* 9, 299-345

Den Hollander, J., (1975) The phenology and habitat of the species of the subgenus *Tipula* Linnaeus in the Netherlands (Diptera, Tipulidae). *Tijdschrift voor Entomologie* 118, 83-97

Downes, J.A. (1969) The swarming and mating flight of Diptera, *Annual Review of Entomology* 14, 271- 298.

Dunnet, G. M. (1955) The breeding of the starling *Sturnus vulgaris* in relation to their food supply. *Ibis* 98, 220-230

Dunnet, G. M. (1956) The autumn and winter mortality of Starlings *Sturnus vulgaris* in relation to their food supply, *Ibis* 98, 220-230

Dunnet, G.M and Patterson , I.J. (1968) The rook problem in the north-east Scotland. in: *The problem of Birds as pests.* (Eds R. K. Murton and E. N. Wright). London Academic Press pp. 119-139

Feare, C. J. (1984) *The starling.* Oxford; Oxford University Press.

Fleming, C. C. and Dolan, S. (1986) The identification of cyst nematodes using agarose isoelectric focussing and silver staining. *Record of Agricultural Research* 34, 47-52

Freeman, B.E. (1966) Cold hardiness and resistance to dessication in Tipulinae larvae. *ARC Conference of Workers on leatherjackets. ARC 28A/67*

French, N. (1969) Assessment of leatherjacket damage to grassland and economic aspects of control. *Proceedings of the 5th British Insecticide and Fungicide Conference.* pp. 511-521

Gair, R , Jenkins, J. E. E and Lester, E. (1983) Wheat Pests. *in Cereal Pests and Diseases*, Farming Press Ltd. pp. 37-43

Golightly, W. H. (1967) Control of *Tipula* spp. larvae. *Plant Pathology (Supplement to 16(1)),* 33-35

Griffiths, C., Carter, J.B. and Overend, J. (1984) *Phaonia signata* (Meigen) (Diptera:Muscidae) larvae predatory upon leatherjackets, *Tipula paludosa* (Meigen) (Diptera; Tipulidae) larvae. *Entomologist's Gazette* 35, 53-56

Jepson, L.R., Mc Murtry, J.A., Mead, D.W., Jesser, M.G. and Johnson, H.G. (1975) Toxicity of citrus pesticides to some predaceous phytoseeid mites. *Journal of Economic Entomology* 68, 707-710

Jones, F.G.W and Jones, M.G. (1984) Flies - Diptera In: *Pests of Field Crops*, Edward Arnold pp. 140-169

Kell G.V. and Blackshaw , R.P. (1988) The influence of agricultural practices on leatherjacket populations in Northern Ireland. *Proceedings of the 12th General Meeting of the European Grassland Federation* pp. 504-508

Keller, S., (1977) Uber zwei Entomophtorosen von *Tipula paludosa* Meig. *Mittelungen-Schweitzerische Entomologische Gesselschaft* 50, 277 -284.

Keller, S., (1978) *Entomophthora gigantea* sp. nov and *E. caroliniana* (Thaxter) com. nov., two pathogens of *Tipula paludosa* Meig. *Sydowia, Annales Mycologici Ser 2* 31, 87-93

Kluyver, H. N., (1933) Bijdrage tot de biologie en de ecologie van den spreeuw *Sturnus vulgaris vulgaris* L.) gedurende zijn voort plantingstijd. *Verslagen en Meddeedelingen Plantenziektenkundige Dienst Waageningen* 69, 1-45

Lam, A.B.Q. and Webster, J.M. (1971) Morphology and biology of *Panagrolaimus tipulae* n. sp. and *Rhabditis tipulae* n. sp., from leatherjacket larvae, *Tipula paludosa*. *Nematologica* 17, 201-212

Lam, A.B.Q. and Webster, J.M., (1972) Morphological characteristics for differentiating larval instars of leatherjackets, *Tipula paludosa* (Diptera: Tipulidae) *Canadian Entomologist* 104, 899-902

Laughlin, R. (1958) The rearing of craneflies (Tipulidae). *Entomologica Experimentalis et Applicata* 1, 241-245.

Laughlin., R. (1960). Biology of *T. oleracea* L. Growth of the larvae. *Entomologica Experimentalis et Applicata* 3, 185-197

Laughlin, R. (1967) Biology of *Tipula paludosa*; Growth of the larvae in the field. *Entomologica Experimentalis et Applicata* 10, 241-245

Mc Cracken, D., I. (1990) *Factors affecting the availability of invertebrate food for the Chough*, Pyrrhocorax *L* Ph.D Thesis, University of Glasgow

Mc Cracken, D.I., Foster, G. N., Bignal, E. M. and Bignal, S. (1992) An assessment of chough, *Pyrrhocorax pyrrhocorax,* diet using multivariate analysis methods. *Avocetta* 16, 19-29

Mc Cracken, D.I., Foster, G.N and Kelly, A. (1995) Factors affecting the size of leatherjacket (Diptera: Tipulidae) populations in pastures in the west of Scotland. *Applied Soil Ecology* 2, 203-213

Mc Kinlay R. G. (1992) Pests of Cruciferous Crops in *Vegetable Crop Pests* Macmillan Academic and Professional Ltd pp. 124-133

Mackinnon, D. L. (1912) Protists parasitic in the larva of the cranefly, *Tipula sp : Parasitology* 5, 175-189

Maercks, H. (1953) Uber den Massenwechsel von *Tipula paludosa* Meig., in den Jahren 1918-1953 und seine Abhangigkeit von der Witterung. *Nachrichtenblatt Deutschen Pflanzenschutzdienstes* 4, 129-133

Meats , A. (1967) The relation between soil water tension and rate of development of the eggs of *T. oleracea* and *T. paludosa.* (Diptera : Nematocera) *Entomologia Experimentalis et Applicata* 10, 394-400

Meats, A. (1970) Susceptibility of the leatherjackets *Tipula oleracea* and *T. paludosa* to soil flooding. *Annals of Applied Biology* 65, 25-30.

Meats, A. (1972) The effect of soil flooding on the survival and development of the eggs of *Tipula paludosa* L and *Tipula oleracea* Meigen (Diptera: Tipulidae). *Journal of Entomology* 46, 99-102

Miles, H.W. (1921) Observations on the insects of grasses and their relation to cultivated crops. *Annals of Applied Biology* 8, 170-184.

Milne, A. (1966) Work on leatherjackets by the A.R.C. Unit of Insect Physiology. *Minutes of Conference of Workers on Leatherjackets. A.R.C. 28A/67*

Milne, A., Laughlin , R. and Coggins, R.E. (1965) The 1955 and 1959 population crashes of the leatherjacket, *Tipula paludosa* Meigen in Northumberland. *Journal of Animal Ecology* 34, 529- 534

Morison, G. D. (1951) Insects and other pests of 1950. *Transactions of the Royal Highland Agricultural Society of Scotland*, 1-43.

Mowat, D. J. and Jess, S. (1986) The control of leatherjackets, *Tipula paludosa* Meig. , in grassland by early application of insecticide. *Grass and Forage Science* 41, 27-30

Mowat, D. J and Shakeel, M.A., (1988) The effect of some invertebrate species on the growth of white clover (*Trifolium repens L.*) in the laboratory. *Grass and Forage Science* 43, 405-409

Muller-Kogler, E. (1957) Uber eine Mykose der Larven von *Tipula paludosa* Meig. durch empusa sp. *Zeitschrift fur Pflanzenkranheitent Pflanzenschutzdienstes* 64, 529-534

Muller -Kogler, E. (1958) Eine Rickettsiose von *Tipula paludosa Meig.* durch *Rickettsiella tipulae* nov. spec. *Die Naturwissenschaften* 45, 248

Newbold, J. W. (1981) The control of leatherjackets in grassland by winter pesticide applications. *Proceedings of Crop Protection in Northern Britain 6* pp. 207-211

Paul, V. H. and Rawlinson, C.J. (1992) *Diseases and Pests of Rape*. Verlag Th. Mann. Gelsenkirchen-Buer. p. 132.

Peters, A. and Ehlers, R.U. (1994) Susceptibility of leatherjackets (*Tipula paludosa* and *Tipula oleracea* Tipulidae, nematocera) to the entomopathogenic nematode, *Steirnema feltiae*. *Journal of Invertebrate Pathology* 63, 163-171

Pinchin, R.D. and Anderson, J. (1936) On the nocturnal actvity of Tipulinae (Diptera) as measured by a light trap. *Proceedings of the Royal Entomology Society of London* 2, 69-78

Rayner, J. M. (1975) Chemical control of leatherjackets (*Tipula* spp.) in cereals. *Proceedings of the 8th British Insecticide and Fungicide Conference*, pp. 231-236

Rennie, J. (1916) On the biology and economic significance of *Tipula paludosa.* Part 1 Mating and oviposition, *Annals of Applied Biology* 2, 235-240

Rennie, J. (1917) On the biology and economic significance of *Tipula paludosa.* Part 2 hatching, Growth and Habits of the Larva, *Annals of Applied Biology* 3, 116-137

Rennie, J. (1924) A mermithid parasite of *Tipula paludosa* Meigen. *Proceedings of the Royal Physical Society of Edinburgh* 11, 1-3

Rennie, J., Sutherland, G.H. (1921) On the life history of of *Bucentes (Siphona) geniculata* (Diptera:Tachinidae), parasite of *T. paludosa* (Diptera) and other species. *Parasitology* 12, 199-210

Ricou, G. (1975) Production de *Tipula paludosa* Meig. en prairie en fonction de l'humidite du sol. *Revue d'Ecologie et de Biologie du Sol* 12, 69-89

Sellke, K, (1936) Biologische und morphologische Studien an schadlichen Wiesenschnaken (Tipulidae, Dipt.). *Zeitschrift fur Wissenschaftliche Zoologie* 148, 465-555

Sherlock, P.L. (1979) *Diplocystis tipulae* sp. nov. (Sporozoa; Eugregarinorida) a parasite of *Tipula paludosa* Meigen (Diptera:Tipulidae). *Parasitology* 78, 207-220

Silantjev, I. (1931) Contributions to a monograph of *Tipula oleracea* L *Plant Protection, Leningrad* 7, 29-45.

Smith, K.G.V. (1989) An introduction to the immature stages of British flies. Diptera larvae, with notes on eggs, puparia and pupae. *Handbooks for the Identification of British Insects 10 (14),* 35-143

Stephenson, J.W., Bardner, R. (1976) Slugs in Agriculture. *Rothamsted Experimental Station Report. Part 2* pp. 169-171

Stewart R.M., Kozicki, K.K. (1987) DIY assessment of leatherjacket numbers in grassland. *Proceedings of Crop Protection in Northern Britain* pp. 349-353.

Stich, H.F. (1963) An experimental analysis of the courtship pattern of *Tipula oleracea* (Diptera), *Canadian Journal of Zoology* 41, 99-109

Theobald, F. V. (1913) *Agricultural Zoology.* Blackwood and Sons, Edinburgh. p. 228

Theobald, F. V. (1929) Some notes on injurious insects and other animals in 1928. *Journal of the South-eastern Agricultural College at Wye* 26, 104-116

Theowald , B. (1957) Die Entwicklungsstadien der Tipuliden (Diptera,Tipulidae), insbesondere der westpalaearktischen Arten. *Tijdschrift voor Entomologie.* 100, 195-308

Theowald., B (1967) *Familie Tipulidae (Diptera, Nematocera) : Larven und Puppen.* Bestimmungsbucher zur Bodenfauna Europea, Lief 7 , Berlin

Tucker, G.G. and Cutler, J.R. (1979) A survey of pesticide usage against leatherjackets (Tipula species) in south-west Scotland in 1975. *Pesticide Science* 10, 247-253

Vickerman, G.P. (1988) Farm scale evaluation of the long term effects of different pesticide regimes on the arthropod fauna of winter wheat. In: Greeves, M.P., Greig-Smith, P.W. and Smith, B.D. (eds*), Field Methods for the Environmental Study of the Effects of Pesticides. BCPC Monograph No. 40,* British Crop Protection Council, Farnham, UK pp. 127-35

Vlug, H.J. (1990) Feeding behaviour of Tipulid larvae on grass. *Mededelingen van de Faculteit Landbouwweten - schappen Rijksuniversiteit Gent* 55, 545-547

Waalwijk, C., Dullemans, A., Weigers, G. and Smit, P. (1992) Toxicity of *Bacillus thuringiensis* variety *Israelensis* against Tipulid larvae. *Journal of Applied Entomology* 114, 415-420

Walters, K.F.A. and Lane, A. (1992) Incidence of pollen beetles in winter oilseed rape and evaulation of thresholds for control. *Brighton Crop Protection Conference - Pests and Diseases* pp. 545-551

White, J. H. (1963) Observations on leatherjacket population assessment, forecasting and control measures. *Proceedings of the 2nd British Conference on Insecticides and Fungicides, Brighton.* pp. 51-58.

White, J. H., French, N. (1968) Leatherjacket damage to grassland. *Journal of the British Grassland Society* 23, 326-329

Whytock, G.P. and Carneigie, H.M. (1990) A Survey of Weeds in Oilseed Rape Fields in North-East Scotland. *Proceedings Crop Protection in Northern Britain.* pp. 307-312