PHYSIOLOGY AND BIOCHEMISTRY OF SEED GERMINATION IN *Striga hermonthica* Del. (Benth.).

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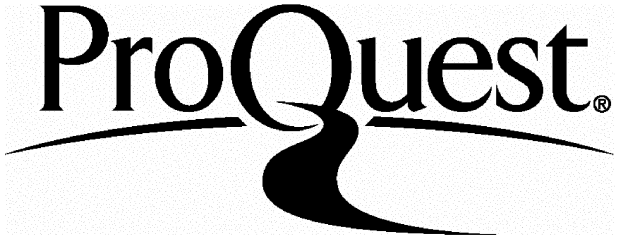

ABSTRACT

Seeds of the plant parasitic angiosperm *Striga hermonthica* (Del.) Benth. possess a specialised dormancy mechanism operating in three sequential phases. Seeds require an after-ripening period followed by an extended period of imbibition (conditioning) before they acquire the potential for maximum germination. However, this potential is not realised unless the seed is in contact with compounds present in the root exudates of the host plant. Although a variety of compounds can substitute for the host derived signal the mechanism through which these act is unknown. This thesis examines the physiological and biochemical nature of both the imbibition and germination phases in the life-cycle of *S. hermonthica.*

An inhibitor of ethylene biosynthesis, aminoethoxyvinyl glycine (AVG) was found to inhibit germination while addition of an intermediate in ethylene biosynthesis, 1-aminocyclopropane-1-carboxylic acid (ACC) was found to override this inhibition and to act as a substitute for the host derived signal. 2,5-norbornadiene (NDE), an inhibitor of ethylene action, also inhibits germination. Ethylene is rapidly produced by *Striga* seeds after treatment with host root exudates. These results are consistent with a model for *Striga* seed germination in which host derived signals and other compounds act by eliciting the synthesis of ethylene and in which ethylene itself initiates the biochemical changes leading to germination. Further studies using inhibitors of the ethylene biosynthetic pathway together with the cytokinin-active urea derivative thidiazuron, suggest that stimulation of the enzyme ACC synthase is necessary for natural host stimulated germination. Analyses of the effect of various combinations of stimulants and

inhibitors on the germination of *S. hermonthica* prior to conditioning suggest that this phase is necessary, not for germination itself, but rather for the seed to react to the external host derived stimulus by initiation of ethylene production.

Time-course analysis of polypeptide changes in seeds triggered to germinate by various compounds using 2-D SDS-PAGE allowed certain polypeptides to be identified as important to the germination process and likely to be involved in the primary events of ethylene biosynthesis or action.

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ABBREVIATIONS

ACC - 1-aminocyclopropane-1-carboxylic acid

AVG - aminoethoxyvinyl glycine

cv. - cultivar

f.wgt. - fresh (unimbibed) weight

GA - gibberellic acid

G_{max} - germination percentage at 48 h

h - hour(s)

IAA - indole-3-acetic acid

I_{50} - inhibitor concentration causing 50% inhibition

L - litre

min - minute(s)

NDE - 2,5-norbornadiene

P - probability

PAGE - polyacrylamide gel electrophoresis

PDA - piperazine di-acrylamide

PPO - 2,5-diphenyloxazole

R_i - initial germination rate

SAM - S-adenosyl-L-methionine

S.D. - standard deviation

SDS - sodium dodecylsulphate

S.E. - standard error of the mean

SEM - scanning electron microscope/microscopy

s.H_2O - sterile double distilled deionised water

S.T.P - standard temperature and pressure

CHAPTER 1 - INTRODUCTION

1.1 Parasitic Angiosperm Seeds

Forming 1% of the total number of all flowering plants, parasitic angiosperms are a diverse group of organisms. With individual species totalling more than 3000, distributed within 17 plant families, parasitic plants are found throughout the Old and New Worlds, (Kuijt, 1969).

Parasitic plants rely on their host for some, if not all, of their nutrients. Attaching to either roots or shoots, they capture water and solutes from the host via the haustorium, a novel organ common to all parasitic angiosperms. While the haustorium has a similar physiological function in different species it is a highly variable organ both morphologically and anatomically.

Parasitic angiosperms are frequently divided into one of two groups, holoparasitic or hemiparasitic, although only at the extremes of each are they truly distinct. Holoparasites depend almost entirely on their host for their nutritional requirements. Lacking chlorophyll and thus unable to photosynthesise, they are all obligate parasites. Hemiparasites can be classified, according to their degree of host dependence, as either facultative or obligate parasites. Facultative hemiparasites are capable of surviving and setting seed in the absence of a host although most are parasitic in their natural habitats, e.g. *Rhinanthus, Bellardia, Euphrasia, Odontites.* Obligate hemiparasites are partly dependent on their hosts for their nutritional requirements while being completely dependent on their host for survival. All hemiparasites are chlorophyllous and were traditionally thought only to rely on their host for water and minerals. However, there is frequently

substantial movement of carbon from host to parasite (Press *et al.*, 1987).

Parasitic angiosperms may be further classified by their site of attachment to the host. Shoot parasites include the holoparasitic dodders - *Cuscuta* spp., belonging to the family Convolvulaceae, the evolutionary distinct scrub dodders, *Cassytha* spp., belonging to the family Lauraceae, and hemiparasites such as mistletoes of the two families Loranthaceae and Viscacea which include the well-known Christmas mistletoe, *Viscum album* L. and its North American counterpart *Phroradendron serotinum* L.. Root parasites range from the holoparasitic Orobanchaceae (broomrapes), Balanophoraceae and Rafflesiaceae to the hemiparasitic mistletoe, *Nuytsia spp.* found in Australia, and the largest family of root hemiparasites, the Scrophulariaceae or figworts.

1.1.1 Seed dispersal and germination strategies

Although the divisions described above provide a means of classification according to mode of parasitism, it is of more relevance in the present context, to classify individual parasites by means of their seed dispersal and germination strategies. Using the classification of Kuijt (1969), it is possible to segregate parasites into one of three dissemination/germination strategies although there is some overlap between groupings.

1.1.1.1 Large seeds

The least specialised strategy involves the production of a few relatively large seeds, such as those of *Rhinanthus, Cuscuta, Cassytha and Melampyrum.* These seeds contain sufficient reserves to allow extensive radicle growth while

seeking a host. Seed dispersal mechanisms are generally haphazard or as in the case of *Lathraea*, the seeds are expelled violently from the capsule when it dehisces (Beck von Mannagetta, 1930). Germination of *Cuscuta* seeds in the laboratory has frequently entailed treatment with sulphuric acid (Gaertner, 1950) or grinding with glass powder (Walzel, 1952) to overcome seed-coat-imposed dormancy mechanisms. However, these scarification treatments are by no means universal in the *Cuscuta* genus. Some desert species germinate precociously (Kuijt, 1969) while, in *C. campestris* Yunker, germination of a seed generation is spread over several years (Dawson, 1965). These mechanisms are not unique to parasitic angiosperms and are therefore considered unspecialised with respect to non-parasitic angiosperm seeds.

1.1.1.2 Animal dispersal

The second strategy relies on animal dispersal to ensure the parasite contacts a host. Many seeds in this group are surrounded by fleshy tissue often brightly coloured making them very attractive to fruit eating animals. The seed is frequently protected by a tough seed coat which allows passage through the intestinal tract of animals without damage. This method is common in the epiphytic mistletoes, *Phacelaria*, the large seeds of which allow the production of a substantial haustorium for attaching and penetrating the woody host tissue (Kuijt, 1969). As a result of the necessary size of these seeds there is usually only one per flower although the large fleshy fruits of *Prosopanche americana* in the family Hydnoraceae may contain as many as 35 000 minute seeds each (Cocucci, 1965). These large fruits are known to be eaten by baboons, jackals,

foxes, porcupines, and in South America by armadillos as well as the local people (Kuijt, 1969). Ants are also known to be involved in the dispersal of parasitic angiosperm seeds. Ants have been observed carrying the seeds of *Mystropetalon* to their nest and fruits have even been retrieved from the nest although without the elaiosome; seeds of *Tozzia* and *Pedicularis* are also known to be dispersed by ants (Kuijt, 1969). In one case it has been suggested that seeds of *Rafflesia* are dispersed by both elephants and ants (Kuijt, 1969). Again, the germination strategies (as opposed to the dissemination strategies) of these parasites are relatively unspecialised.

1.1.1.3. Host stimulated

The third strategy is unique to parasitic plants and involves a highly specialised mechanism by which the parasite seed is stimulated to germinate by chemical triggers derived from the host plant. It is this mechanism with which this thesis is concerned.

All of the Orobanchaceae, four genera of the Scrophulariaceae (*Striga, Alectra, Lathraea and Tozzia*), most Rafflesiaceae, Balanophoraceae and Hydnoraceae require the presence of host-derived chemicals to stimulate germination (Press *et al.*, 1990). The success of this mechanism hinges on the production of vast numbers of minute seeds (350 x 230μm in *Striga hermonthica*) which are dispersed by wind and water. Many of the species within this grouping are serious agricultural pests, particularly in the Developing Countries where they attack and devastate cereal and legume crops. Such parasitism results in reduction of shoot growth, severe wilting and chlorosis which contribute to yield reductions

of up to 100% in the case of *Sorghum* infected with *Striga hermonthica* (Del.) Benth. (Doggett, 1965).

The vast majority of parasitic plant research has been conducted on agriculturally important genera, most commonly these are obligate parasites. *Alectra vogelii* Benth. is a serious parasite of legume crops in Africa, while other *Alectra* species can attack tobacco, sunflower and other crops (Wild, 1954). One genera of the Orobanchaceae, *Aeginetia,* is known to attack sugarcane, sorghum and millet. Facultative parasites of the Scrophulariaceae, such as species of *Rhinanthus* and *Odontites,* have caused limited growth losses in some areas in the past but their importance has never reached that of *Striga* and *Orobanche*.

1.1.2 After-ripening and dormancy

In common with the majority of non-parasitic angiosperm weeds most parasitic weed seeds have some form of dormancy mechanism. Where specialized (host-induced) germination strategies have evolved the seeds must first undergo a period of after-ripening. Defined as any internal changes which take place after seed shed which lead to germination or increased germination, after-ripening is a period of dormancy during which germination will not occur (or occurs only to a limited extent) even under optimal conditions (Bewley and Black, 1982). This period, which generally lasts for up to 6 months, is a common feature of both *Striga* and *Orobanche*, the most economically important genera. Very little is known concerning the nature of the after-ripening period or the changes which lead to the release from this dormancy mechanism.

Once after-ripened, seeds of *Striga* and *Orobanche* require a period of

imbibition at temperatures ranging from 20 to 35°C for 2 to 14 days before they acquire the potential to germinate in response to host-derived chemical triggers. This imbibition period was first characterised by Brown and Edwards (1944), who coined the term "pretreatment". The terms preconditioning, pre-exposure and conditioning have also been used for the same process. The seeds should now be considered as quiescent, since germination will occur under favourable conditions, i.e. the presence of an external chemical stimulus. Although the optimum temperature for, and length of, conditioning vary between species and even between different samples of the same species, the conditioning process is an absolute requirement for many taxa within the families Scrophulariaceae and Orobanchaceae (*Striga asiatica* (= *lutea*): Brown and Edwards, 1944; *Alectra vogelii* Benth.: Botha, 1948; *S. hermonthica*: Vallance, 1950; *S. euphrasioides* (Vahl.) Benth.: Rangaswamy and Rangan, 1966; *O. minor* Smith and *O. crenata* Forks.: Edwards, 1972; *Sopubia delphinifolia* G. Don.: Shivanna and Rangaswamy, 1976; *Striga densiflora* Benth.: Reid and Parker, 1979; *Orobanche ramosa*: Saghir, 1986).

Much research has been carried out on the conditioning process, with most workers concentrating on the length of the conditioning period and the optimum temperature for conditioning (Brown and Edwards, 1946; Botha, 1950; Vallance, 1950, 1951b; Reid and Parker, 1979). While it is not known what changes occur during the conditioning process, there is evidence, in the case of *S. asiatica,* for the role of endogenous germination inhibitors (Williams, 1959; Kust, 1966). The results of Hsiao *et al.* (1979) demonstrated the interactions among seeds during conditioning: they found that a greater number of seeds per dish or

a smaller volume of water increased subsequent germination on stimulation by strigol (a stimulant collected from cotton, see later), results which seem to contradict the inhibitor theory. However, these correlations were only apparent at threshold strigol concentrations.

Seeds of *Aeginetia indica* L. are also dormant following seed shedding. Dormancy in these seeds can be broken by treatment with sodium hypochlorite, exposure to low temperatures (3-5°C) for several days (stratification), or a short exposure to higher temperatures (15 min at 50°C) (French and Sherman, 1976). Whether this dormancy mechanism can be equated with the after-ripening period required by species of *Striga* and *Orobanche* or with the quiescent period following after-ripening is unclear. Kusano (1908) found that *Aeginetia* required the presence of a host, or roots of certain non-hosts before germination would occur whereas French and Sherman (1976) claim that host/non-host presence is unnecessary. However French and Sherman treated seed in their experiments with sodium hypochlorite as standard, a procedure which itself could result in germination (see 1.1.5), or may potentiate the effects of other treatments. After initiation of germination in *Aeginetia* the presence of a root of a host (e.g. sugarcane, *Saccharum officinarum* L.) or non-host (e.g. pea, *Pisum sativum* L.) stimulates formation of fine tendril-like protuberances which function in host attachment (Kusano, 1908; French and Sherman, 1976).

Stratification has also proved effective in other species such as *Aureolaria pedicularia* (Musselman, 1969); *Castilleja coccinea* (Malcolm, 1966); *Euphrasia* (Yeo, 1961); *Odontites verna* (Govier and Harper, 1965); *Rhinanthus cristagalli* (Vallance, 1952); and *Melampyrum lineare* Desr. (Curtis and Cantlon, 1963). In

M. lineare, an annual root hemiparasite, germination requires two steps: firstly, activation at approx. 20°C followed by exposure to low temperatures (4°C) for 6 to 8 weeks (Curtis and Cantlon, 1963). Activation at 20°C results in up to 40% of a seed batch germinating after subsequent cold treatment, while treatment with gibberellic acid induces up to 100%. Although the ecological importance of this mechanism is understood - spreading germination of the annual seed crop over a number of years (germination occurring after each winter) increases the likelihood of the seed encountering a suitable host - the biochemical reasons for the embryo's additional requirements for endosperm hydrolysis (ie. the requirement for exogenous GA to maximise germination) and its cold dependence remain unknown (Curtis and Cantlon, 1968).

If the period of conditioning is extended greatly beyond the optimal time, the seeds of *Striga hermonthica* have been reported to enter a period of "wet dormancy" (Vallance, 1950) which can be alleviated if the seeds are dehydrated and conditioned once more. Wet dormancy is an example of secondary dormancy as opposed to the primary or innate dormancy mechanisms mentioned above. Secondary dormancy is often induced, as in this case, when seeds are given all but one of the conditions necessary for germination (Mayer and Poljakoff-Mayber, 1989). Recently the onset of secondary dormancy in *S. hermonthica* has been reinvestigated by Pieterse *et al.,* (1984), who also found that germination decreases after a prolonged period of conditioning, although the period required was much greater than that reported by Vallance (1950) (10-14 weeks compared with 3-4 weeks). Also in contrast to Vallance's results, Pieterse *et al.* found that drying and then reconditioning resulted in low germination percentages in only

two out of eight samples. Hsiao *et al.* (1987) found no evidence of wet dormancy after conditioning *S. asiatica* for 56 days. Although this may be an inter-species difference it could be due to the age of seed populations used. Vallance (1950) noted a greater reduction in germination in 6-year-old seed compared with 1-year-old; Hsiao *et al.* used 1-year-old seed.

1.1.3 Host-derived germination stimulants

As mentioned in 1.1.1.3, the seeds of species in almost all genera of root-parasitic plants require stimulation of germination by chemicals exuded from the host plants' roots. This unique phenomenon was first demonstrated by Vaucher (1823) for *Orobanche* and *inter alia* by Garman (1903) with *Orobanche minor* Smith, by Kusano (1908) with *Aeginetia indica* (see 1.1.2) and by Heinricher (1917) with *Tozzia* and *Lathraea*. Pearson (1911) noted that germination of *Striga* "only occurs when the seed lies very close to - probably in actual contact with - a root on which the rooibloem (*Striga*) will grow". However, Saunders (1933), working on *S. asiatica*, was the first to demonstrate that actual contact between parasite and host root was not required, by stimulating seeds to germinate with a water-soluble exudate from maize roots. This conclusion was also reached by Brown and Edwards (1944) using a different experimental design involving *S. asiatica* and exudate from intact seedlings of *Sorghum vulgare*.

Many different compounds are released by host and non-host plants, which are active in stimulating the germination of root-parasitic angiosperm seed, and the ability of each compound in the mixture to stimulate germination can

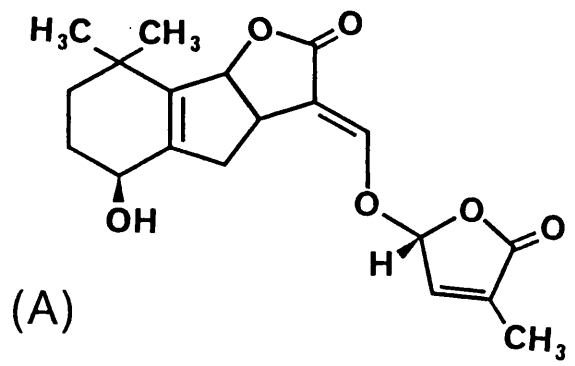
vary with different parasite species (Sunderland, 1960; Cook *et al.*, 1972; Visser and Botha, 1974; Visser, 1975; Hauck *et al.*, 1992; Muller *et al.*, 1992). Many workers have attempted to identify the germination stimulants exuded from host plants' roots, but have had considerable difficulty for two main reasons. Firstly, host-derived germination triggers are active at very low concentrations (down to 10^{-15} mol m^{-3} of soil solution) (Press *et al.*, 1990). Secondly, these compounds are extremely labile, being very sensitive to pH changes and oxidation.

The first naturally occurring germination stimulant was reported from root exudates of cotton by Cook *et al.*, (1966). Six years later the stimulant, identified as a sesquiterpene, was given the trivial name, strigol (Cook *et al.*, 1972) (Fig. 1.1A), but it was a further 2 years before total synthesis of the molecule was accomplished (Heather *et al.*, 1974, 1976) and yet a further 11 years before the absolute structure was established (Brooks *et al.*, 1985). The breakthrough by Cook and his colleagues offered the possibility of using chemical induction of the germination of the parasite as a means of clearing fields of *Striga* infestation.

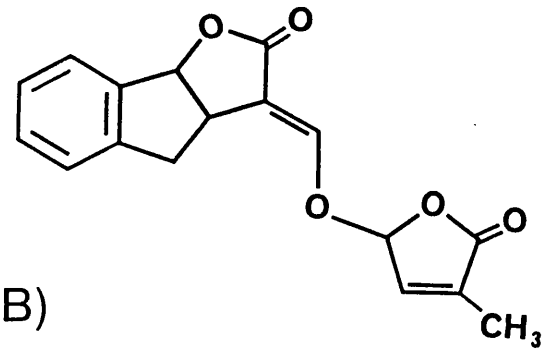
Strigol is active at concentrations as low as 10^{-9} mol m^{-3} in soil solution and it was not long after its identification that structure-activity studies were conducted which led to the synthesis of a number of analogues (termed GR compounds after G. Rosebury) and precursors (Johnson *et al.*, 1976, 1981). Most of the compounds tested were more active in stimulating germination of *Orobanche* than of *Striga* seed. Many synthetic lactones that were active in stimulating germination of *Orobanche ramosa* had no stimulatory effect on *Striga hermonthica*; there was also a disparity in the effects of certain lactones on germination of *S. hermonthica* and *S. asiatica* (Johnson *et al.*, 1976).

Figure 1.1

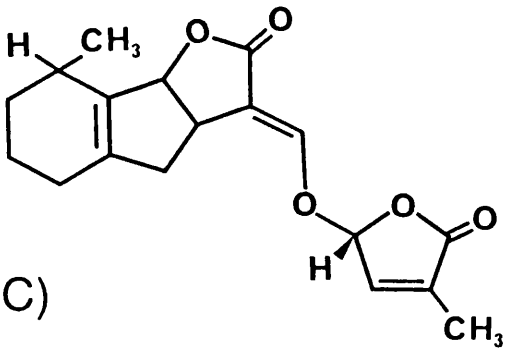
Germination stimulants - (**A**) Strigol; (**B**) GR-24; (**C**) Sorgolactone; (**D**) Sorgoleone-358; (**E**) Cowpea stimulant C-3; (**F**) Thidiazuron.


H3C
CH3
O
O
OH
O
O
O
H
CH3

(A)


O
O
O
O
O
CH3

(B)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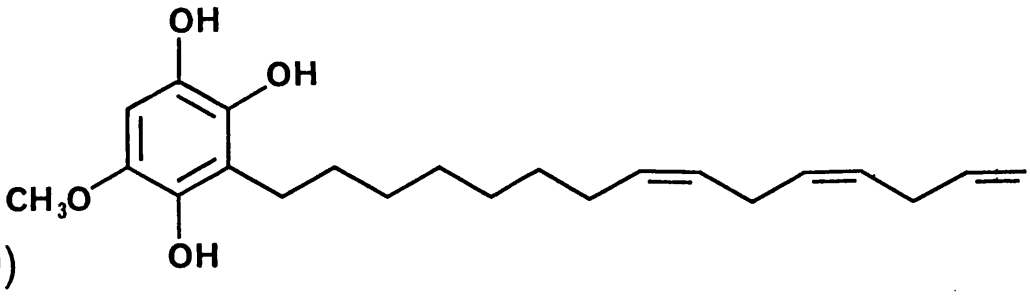
CH3
O
O
O
O
O
H
CH3

(C)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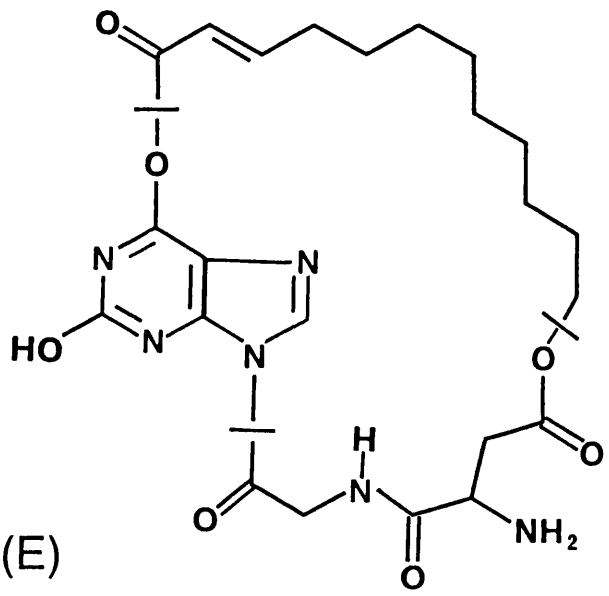
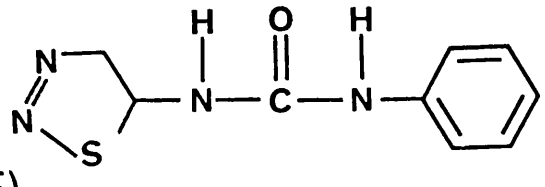
OH
CH3O
OH

(D)


O
O
N
N
HO
N
N
O
O
H
N
O
O
NH2

(E)


H
O
H
N
N
C
N
N
S

(F)

One of the most active analogues to date is GR-24 (Fig. 1.1B). Recently, Zwanenburg and co-workers have revived the structure-activity approach (Zwanenburg *et al.*, 1986; Mhehe, 1987). Simplification of the molecule to the ring framework and the use of different substituted groups, plus knowledge of the molecular shape of strigol and bio-isomerism suggest that the linkage between the two lactones is vital for biological activity.

In addition to strigol and its analogues, many other sesquiterpene lactones are known to stimulate germination of *S. asiatica*. Comparison of the stimulatory activity of ten germacranolides indicated that it may be the medium-ring skeleton and the lactone ring of these compounds which are important to this activity (Fischer *et al.*, 1990). A second naturally occurring germination trigger has been identified from the root exudates of *Sorghum vulgare*. Hydrophobic droplets on *Sorghum* root hairs were first realised to be *Striga* germination stimulants by Netzly and Butler (1986). These droplets contain a number of *p*-hydroquinones present in either oxidised or reduced form (dihydroquinone) (Chang *et al.*, 1986; Netzly *et al.*, 1988). After extraction with dichloromethane and separation by HPLC two major components were identified. The biological activity was related to the concentration of only one of these two compounds which was given the name sorgoleone-358 (2-hydroxy-5-methoxy-3-[(8'Z,11'Z]-8',11',14'-pentadecatriene]-*p*-dihydroquinone (Fig. 1.1D). This compound is very unstable and is rapidly oxidised to the quinone form although reduction back to the dihydroquinone has been shown using tin amalgam or zinc dust. Sorgoleone-358 is active at higher concentrations than strigol (10^{-4} mol m^{-3} compared with 10^{-9} mol m^{-3}) while being much more labile. Recently, germination stimulants have

been isolated from the host *Sorghum bicolor* (Hauck *et al.*, 1992). This complex mixture of stimulants includes two which are more active in stimulating the germination of both *Striga asiatica* and *S. hermonthica* while another compound is more active in stimulating the germination of *S. gesnerioides* (Willd.) Vatke and *Alectra vogelii*. Preliminary identification of the most active component stimulating germination of *S. hermonthica* and *S. asiatica*, given the name sorgolactone, suggests a structure similar to that of strigol (Fig. 1.1C) (Hauck *et al.*, 1992). A fourth compound, identified from the root exudates of cowpea, *Vigna unguiculata* Walp., has been implicated in the germination of *S. gesnerioides* and *A. vogelii* (Herb *et al.*, 1987; Visser *et al.*, 1987): this cyclic molecule consists of three parts, a xanthine, an unsaturated C_{12}-carboxylic acid and a dipeptide of aspartic acid and glycine (Fig. 1.1E).

The germination trigger, sorgoleone-358, identified by Chang *et al.* (1986), has been shown to function in the characterization of host distance and it was suggested to be responsible for the chemotropic growth response of the parasite radicle (Fate *et al.*, 1990). When *S. asiatica* seeds were placed at distances parallel to the root of a 4-day-old *Sorghum bicolor* seedling in an agar plate, maximum germination occurred within a radius of 5mm, which is equal to the maximum length of the *Striga asiatica* radicle (Fate *et al.*, 1990). Imaging of the hydroquinone with methylene blue showed a clear reduced zone detectable after several days, equilibrium being reached at 5mm after 5 days. Although establishment of a steady-state gradient can provide information concerning host presence and spatial distance, it is unknown to what extent this is maintained in the soil (Fate *et al.*, 1990). Williams (1961a,b, 1962), demonstrated the influence

of host-derived chemical signals on the morphology of the germinating parasite seed and showed that not only germination but morphology varied with proximity to the host root. In the zone nearest the host root ($\leq$ 4mm), the rate of extension growth was very low, the parasite radicle undergoing considerable lateral expansion together with the production of root hairs. At this distance, growth of the *S. asiatica* radicle was positively chemotropic although not all seedlings grew towards the host root. As distance from the host root increased, extension growth of the parasite radicle increased with reduced lateral expansion and root hair production. Increased production of root hairs and the redirection of cellular extension at the root meristem from a longitudinal to radial dimension are now known to be typical features in the change from a developmental mode to a parasitic mode of growth in root-parasites (Riopel and Vance Baird, 1987; Smith *et al.*, 1990).

1.1.4 Substitute germination triggers

Apart from strigol, its GR analogues and host-derived stimulants, many other chemicals are known to induce germination of root-parasitic angiosperms. Most of these compounds are known plant growth regulators, having similar effects on non-parasitic angiosperm seeds.

1.1.4.1.Ethylene

The effects of ethylene on the germination of angiosperm seeds has been well documented (Toole *et al.*, 1964; Abeles & Lonski, 1969; Ketring & Morgan, 1969; Taylorson, 1979). Egley and Dale (1970) were the first to

discover that ethylene could substitute for the host-derived chemical stimulus in the germination of conditioned seeds of *S. asiatica*. Ethylene has now been shown to cause germination of *S. hermonthica* (Bebawi and Eplee, 1986) and *Orobanche ramosa* (Chun *et al.*, 1979) while *S. gesnerioides* shows a limited response (A. Antwi, D.C. Logan, G.R. Stewart unpublished). Ethylene has been used on a substantial scale to trigger "suicidal" germination (germination in the absence of a host) as part of a *Striga* eradication program in the USA. Injecting ethylene into infected soil can result in germination of conditioned *S. asiatica* of up to 90% at depths up to 30cm and at horizontal distances up to 70cm from the point of injection (Witt and Weber, 1975).

1.1.4.2 Cytokinins, Auxins and Gibberellic acid

Cytokinins have also been shown to cause germination of *Striga* seeds (Worsham *et al.*, 1959; Igbinnosa and Okonkwo, 1992). Using kinetin, benzyladenine and other 6-substituted aminopurines, Worsham *et al.* (1959) compared the efficacy of these compounds with that of a natural stimulant solution obtained from maize seedlings. At all but threshold concentrations, seeds stimulated to germinate by 6-substituted aminopurines were morphologically different from those that germinated naturally. Hypocotyls were much reduced, consisting of a bulbous mass of small unelongated cells with numerous root hair-like protuberances, similar to the morphological changes caused by haustorial initiation; induction of haustoria by benzyladenine has also been demonstrated for *Cuscuta spp.* (Ramasubramanian *et al.*, 1988). Kinetin and zeatin stimulated germination of conditioned and non-conditioned *S. gesnerioides* seeds while

benzylaminopurine was much less effective; all cytokinin treatments resulted in the radicles of germinated seeds being short and swollen (Igbinnosa and Okonkwo, 1992). Treating with kinetin *S. asiatica* seedlings that have been stimulated with host root exudate causes a similar morphological condition with a greater effect at lower concentrations (Williams, 1961b). The role of cytokinins in host root exudate stimulated germination is unknown. Treatment of *S. asiatica* with either IAA or GA_3 failed to induce germination (Egley, 1972). GA_3 causes germination in several species of *Orobanche spp.* in the absence of host-derived stimulus (Pieterse, 1979; Chun *et al.*, 1979). Incubation with IAA during both conditioning and at the same time as treatment with host-derived stimulant was found to increase germination of *O. aegyptiaca* Pers. (Kumar and Rangaswamy, 1977) although stimulation of germination by IAA in the absence of host-root exudate does not occur.

1.1.4.3 Coumarin

Coumarin, generally though of as a germination inhibitor has also been found to stimulate germination of *S. hermonthica* seed (Worsham *et al.*, 1962). Of the many coumarin derivatives tested only two caused any stimulation of germination - hydroxycoumarin and scopoletin.

1.1.4.4 Brassinosteroids

Brassinosteroids have been shown to act synergistically with strigol, kinetin and the ethylene producing compound, ethephon in stimulating germination of *S. asiatica,* with no germination as a result of brassinolide

treatment alone (Takeuchi *et al.*, 1991). In addition, conditioning in brassinolide, as opposed to distilled water, resulted in a greater final germination percentage following stimulation with sorghum root exudate (Takeuchi *et al.*, 1991).

1.1.5 Non-growth regulators

Other compounds which are not found in plant root exudates have been shown to cause germination of *S. asiatica*. These include the substituted urea compounds, thiourea and allylthiourea (Brown and Edwards, 1945) whose significance as natural stimulants is unknown. Oxidising agents such as sodium hypochlorite, calcium hydroxide and some halogens have been tested as germination stimulants (Egley, 1972; Hsiao *et al.*, 1981). Sodium hypochlorite caused germination of *Alectra vogelii* seeds in the absence of any conditioning period, although with conditioning, germination rose to 81-91 % (Okonkwo and Nwoke, 1975). French and Sherman (1976) were also able to induce germination of *Aeginetia indica* using sodium hypochlorite. In addition, sodium hypochlorite can slightly stimulate the germination of conditioned and non-conditioned *S. gesnerioides* seeds and at low concentrations acted synergistically with kinetin, ethylene and root exudate treatments (Igbinnosa and Okonkwo, 1992).

1.1.6 Light

The effect of light on the germination of parasitic angiosperms is primarily determined by the nature of their parasitism. Root parasites of the Scrophulariaceae and Orobanchaceae which germinate in the absence of host derived stimulants, such as *Castilleja coccinea* (Malcolm, 1966), *Sopubia*

delphinifolia G. (Don.) (Shivanna and Rangaswamy, 1976) and *Buchnera hispida* Buch. (Okonkwo and Nwoke, 1974) are stimulated to germinate by light. Sahai and Shivanna (1982) suggest that, since the food reserves of these small seeds are limited, they must germinate very near the soil surface to enable quick emergence and onset of photosynthesis. Parasites with a requirement to be close to the hosts' roots to allow stimulation of germination by host-derived chemicals do not require light for germination. Germination of the seeds of *Striga asiatica* (Worsham *et al.*, 1964) and *Aeginetia indica* (French and Sherman, 1976) was inhibited by light, although germination of water-washed seeds of *S. euphrasioides* was stimulated by daylight (Rangaswamy and Rangan, 1966). The effects of light on host-stimulating germinating seeds of *S. asiatica* are similar to those caused by treatment with cytokinins, notably reduced extension growth of the radicle, increased diameter, production of root hairs and initiation of the haustorium (Williams, 1961b); because of the need for *S. asiatica* seeds to be in proximity to a host root for germination these effects are unlikely to have any significance in nature.

1.2 Ethylene - Biosynthesis and Action

The plant growth regulator ethylene has been implicated in many aspects of plant growth, development and senescence. The earliest reports of ethylene action on plants concerned the effects of illuminating gas on the growth of etiolated pea seedlings (Neljubov, 1901), while Cousins (1910) was first to show that oranges produce a gas capable of causing the ripening of bananas. With the advent of ethylene measurement by gas chromatography workers were able to

show that ethylene is produced by essentially all higher plant parts and that it is involved in processes ranging from root and leaf growth to ripening and senescence (Abeles, 1973).

As mentioned above (1.1.4.1), stimulation of germination by ethylene is well documented (Abeles, 1973; Ketring and Morgan, 1972) as is the ability of exogenously applied ethylene to break dormancy (Vacha and Harvey, 1927; Toole *et al.,* 1964). Ketring and Morgan (1969) were the first to suggest that seeds which respond to exogenous ethylene produce ethylene endogenously during after-ripening and that this endogenous ethylene induces release from dormancy. Although germinating peanut seeds produced increased amounts of ethylene, Ketring and Morgan (1969) did not exclude the possibility that germination promotes ethylene production. The exact role of ethylene in the release from dormancy remains unknown. In many species there exists a complex interaction between ethylene and other factors such as light, CO_2, gibberellins, cytokinins and ABA (Ketring and Morgan, 1972; Olatoye and Hall, 1972; Katoh and Esashi, 1975a,b; Karssen, 1976; Taylorson, 1979).

Lieberman and Mapson (1964), were the first to demonstrate that methionine could act as a precursor of ethylene synthesis in a chemical system. Two years later methionine was confirmed as a ethylene precursor *in vivo* (Lieberman *et al.,* 1966). The discovery that ethylene production could be inhibited by rhizobitoxine (Owens *et al.,* 1971) and other inhibitors of pyridoxal phosphate mediated enzymes such as canaline, aminoethoxyvinyl glycine (AVG) and amino-oxyacetic acid facilitated the unravelling of the full biosynthetic pathway (Fig. 1.2). Adams and Yang (1977, 1979) identified S-adenosyl-L-

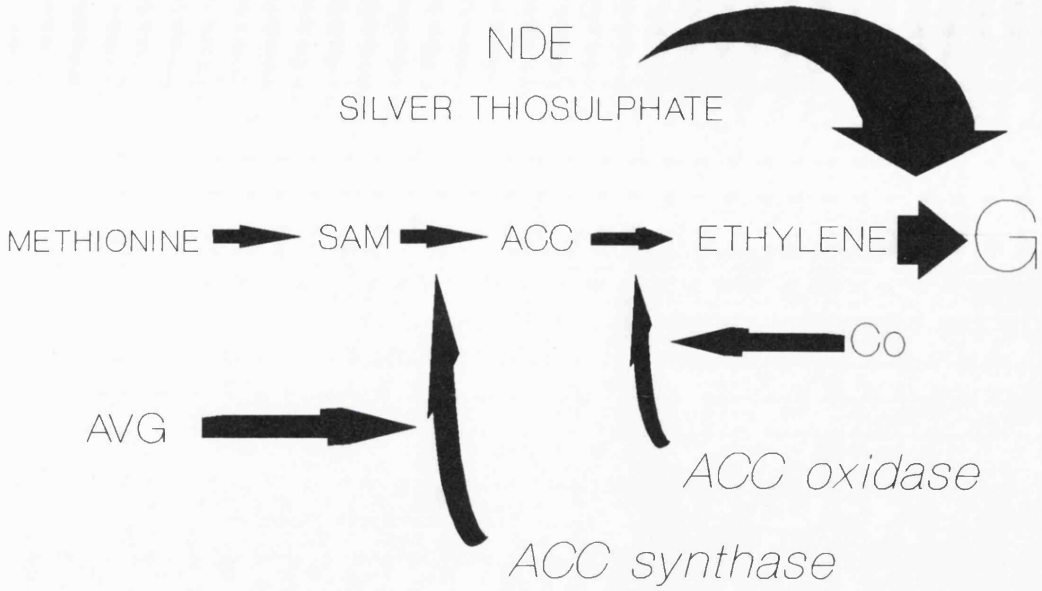


Figure 1.2

Ethylene biosynthesis pathway; SAM, S-adenosyl-L-methionine; ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinyl glycine; NDE, 2,5-norbornadiene.

methionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) as the two additional ethylene precursors in higher plants. AVG was shown to inhibit the conversion of methionine to ACC, while not blocking the conversion of methionine to SAM, or the conversion of ACC to ethylene, thus proving that AVG inhibits the conversion of SAM to ACC and that this conversion is mediated by a pyridoxal enzyme (Adams and Yang, 1979).

Ethylene biosynthesis is also inhibited by cobalt and nickel cations (Lau and Yang, 1976b; Kang *et al.*, 1967), which have been shown to inhibit the conversion of ACC to ethylene (Yu and Yang, 1979). The precise nature of this inhibition is unknown, although it has been suggested that Co^{2+} complexes with sulfydryl groups of proteins (Thimann, 1956; Yu and Yang, 1979).

Ethylene action can be inhibited by inactive structural analogues and by other chemicals believed to interfere with the binding of ethylene to receptor molecules or by occupying the binding site without eliciting a necessary conformational change. The effect of silver (Ag^{2+}) as an ethylene antagonist is almost universal. It has been suggested that the silver ions replace a hypothetical metal ion in the active site of the receptor, thus resulting in non-competitive knock-out inhibition (Beyer, 1976). However, the inhibition of ethylene action by silver can be overcome by high concentrations of ethylene, indicating competitive inhibition (Beyer, 1978). In addition, precipitation of silver ions by acetylene has been found to restore ethylene sensitivity (Beyer, 1978). There are conflicting reports in the literature concerning the inhibitory effect of silver ions on ethylene binding. Goren *et al.* (1984), Sisler *et al.* (1986) and Hall *et al.* (1990) showed that silver inhibited binding *in vivo* while Sanders *et al.* (1991) found no evidence

for this effect and suggested that binding inhibition was an artefact caused by stimulation of ethylene biosynthesis. For the present, the exact mechanism of silver induced inhibition of ethylene action remains to be elucidated.

In addition to inactive structural analogues (eg. trans-2-butene and alkanes), 2,5-norbornadiene (NDE) and related cyclic alkenes such as norbornene and cyclopentene were found to inhibit ethylene mediated responses (Burg and Burg, 1967). Sisler and Pian (1973), reported that cyclic olefins inhibited ethylene action competitively and subsequent studies have verified the ability of NDE to inhibit ethylene action in a wide range of both plant material and physiological conditions (Sisler and Yang, 1984; Bleecker *et al.*, 1987; Wang and Woodson, 1987). Unlike silver ions, 2,5-norbornadiene was found to inhibit both ethylene action and ethylene binding (Sanders *et al.*, 1991).

This thesis describes experiments performed to determine the physiological and biochemical mechanisms which control the seed conditioning and germination stimulation phases in the life cycle of *S. hermonthica.* Manipulation of ethylene biosynthesis and action, coincident with measurement of the ethylene produced, provides evidence for the role of endogenous ethylene in *S. hermonthica* germination. Data are presented which indicate that the conditioning phase is necessary for the seeds to respond to host derived chemical triggers while being unnecessary for germination *per se.* Further comparison between stimulants is provided by analysis of the respiratory activity during conditioning and germination, together with comparison of the pattern of protein changes during germination.

CHAPTER 2 - MATERIALS AND METHODS

2.1 Plant materials and growth conditions

2.1.1 *Striga hermonthica*

Seeds of *Striga hermonthica* (Del.) Benth. were collected from Wad Medani, Sudan, in 1982, 1988 and 1989. In all experiments (unless stated otherwise), seeds were surface-sterilized by washing in a 5% (v/v) solution of sodium hypochlorite (10-14% (w/v) available chlorine stock) for 3 min followed by washing with at least 1 litre (L) of sterile distilled water (s.H_2O). Seeds that were to be conditioned were placed on two sterile circles of Whatman No.1 filter paper, saturated with s.H_2O, in 9cm perspex petri dishes and incubated at 33°C in the dark for the requisite period. Throughout the conditioning period the filter papers were saturated with s.H_2O. Experiments with seeds from 1981 and 1987 utilised non-sterile double distilled deionised water.

Experiments utilized seed collected in 1989 unless stated otherwise. For experiments using seed by weight, fresh weight (f.wgt.) means the weight of mature non-imbibed seed.

2.1.2 Host root exudate collection

Seeds of *Sorghum bicolor* (L.) Moench cv. CSH-1 were surface-sterilized as 2.1.1 for 20 minutes (min) and germinated on moist sterile Whatman No.1 filter paper at 33°C. After 48 hours (h) the germinated seeds were placed on nylon grids suspended over 500cm^3 opaque plastic beakers containing a 20% (v/v) modified Long Ashton solution (Hewitt, 1966), so that the radicles were

immersed in the solution.

Plants were grown in a controlled environment (day temperature 35°C, night temperature 20°C, 16 h day-length, light intensity at bench level of 103 W m^{-2}). Four days later three plants were transferred to 50cm^3 of s.H_2O and incubated at 20-25°C in the dark for 24 h; this solution was used as germination stimulant. Stimulant was prepared freshly for each experiment.

2.2 Chemicals and reagents

All chemicals were purchased from Sigma Chemical Co. LTD. (Poole, Dorset, U.K.), B.D.H. LTD. (Poole, Dorset, U.K.) or Fisons (Loughborough, U.K.) unless stated otherwise and were of analytical grade or higher. NDE was purchased from Aldrich Chemical Co. LTD. (Gillingham, Dorset); 1% ethylene calibration gas in N_2 was purchased from PhaseSep (Deeside, Clwyd, U.K.). Ampholines, ion exchange resin and piperazine di-acrylamide (PDA) were purchased from Bio-Rad Laboratories (U.K.) LTD. (Watford, Herts., U.K.). Bromophenol blue was purchased from Pharmacia-LKB Biotechnology (Milton Keynes, Bucks. U.K.). RBS was purchased from Life Science Laboratories LTD. (Luton, Beds., U.K.). Amino acid standards were purchased from Sigma Chemical Co. LTD. Thidiazuron, as DROPP WP-50 (50% thidiazuron), was a gift from Schering Agrochemicals (Berlin, Germany). GR-24 was a gift from Professor B. Zwanenburg (Department of Organic Chemistry, University of Nijmegen, Nijmegen, Netherlands).

2.3 Measurement of Germination

Test solutions (200μL) and *Striga* seeds (10/well) were added to flat bottomed polystyrene microtitre plates (Sterilin, Middlesex, England.) at time zero and incubated in the dark at 33°C. Each sample was replicated 12 times (i.e. a total of 120 seeds). Incubation media included various concentrations and combinations of host root exudate, the cytokinin-active urea-derivative thidiazuron, the ethylene biosynthesis inhibitors aminoethoxyvinyl glycine (AVG) and Co^{2+} (as $CoCl_2$) and the ethylene biosynthesis intermediate 1-aminocyclopropane-1-carboxylic acid (ACC). When using the strigol analogue GR-24, seeds, 10/vial, together with 400μL of 3.4μM GR-24 were placed in each of 12 (1.95cm^3) silicone rubber/teflon sealed borosilicate glass vials (Life Science Laboratories LTD., Luton, England.) as acetone reacted with the polystyrene microtitre plates; incubation was as above.

For tests involving the ethylene action inhibitor NDE the seeds (10/vial, 12 vials per experiment) together with 400μL exudate were placed in borosilicate glass vials as for GR-24. Vials were sealed and 10 or 100μL of head-space were removed, followed immediately by injecting an equivalent volume of NDE stock. Vials were transferred to a reciprocal rotator (rotating at one revolution s^{-1}) and incubated at 33°C in the dark. NDE stock was prepared by injecting 18μL of NDE liquid into an 8.87cm^3 silicone rubber/teflon sealed borosilicate glass vial, this was left for 1 h at room temperature before use to give a concentration at S.T.P. of 416μL/cm^3.

For measurement of ethylene stimulated germination, seeds (10/vial) were placed in gas-tight vials, as detailed for NDE above, with 400μL s.H_2O replacing

host root exudate. Immediately after sealing, 100 or 250μL of the head-space were removed and 100 or 250μL respectively, of 0.1% (v/v) ethylene were then passed through the water using a gas-tight Hamilton syringe. Incubation was as for NDE treatment. Addition of NDE or ethylene took place at time zero.

Germination counts, based on radicle protrusion, were made at intervals up to 48 h after the final treatment using a low-power stereo microscope (6.7-60x) with both high-intensity epi-illumination and trans-illumination. Controls of s.H_2O were included in all experiments. All experiments were repeated at least twice (i.e. a total of three replicates) except where stated. As germination data were in the form of proportions, mean percentages and standard deviations (S.D.) were calculated from arcsine transformed data (Sokal and Rohlf, 1981).

2.4 Measurement of ethylene - gas chromatography

For each replicate, 30 or 40mg f.wgt. of seed were surface-sterilized and conditioned as 2.1.1, washed with s.H_2O and transferred to an 8.87cm^3 vial containing a final test solution volume of 1.5cm^3. Each vial was placed in the incubator for 1 min for temperature equilibration before being sealed with a silicone rubber/teflon disc. For experiments involving NDE, vials were sealed and 45μL of headspace were removed and replaced immediately with an equivalent volume of NDE stock. Vials were transferred to a reciprocal rotator (rotating at one revolution s^{-1}) and incubated at 33°C in the dark. At intervals, 5cm^3 of the head-space were withdrawn from a vial, using a gas-tight Hamilton syringe, for ethylene measurement. New vials were used for each sampling time. Concentrations were determined by gas chromatography using a Shimadzu GC-

Mini-3 fitted with a Poropak R (80-100 mesh) column and a flame ionization detector. The oven temperature was set at 70°C and the injector at 120°C. Helium was used as carrier gas at $30cm^{-3}$ min^{-1} with air and hydrogen set at flow rates of $300cm^{-3}$ min^{-1} and $30cm^{-3}$ min^{-1} respectively. A standard curve was produced covering the range of ethylene concentrations obtained in preliminary experiments and the response was found to be linear. Peak areas were converted to concentrations using linear regression.

2.5 Low-power photomicrography

Samples of germinated seed were transferred to glass microscope slides in a small drop of incubation medium. Photomicrographs were taken using transmitted white light or green high-intensity epi-illumination to increase contrast.

2.6 SDS-Polyacrylamide gel electrophoresis (PAGE)

2.6.1 Sample preparation

For each sample, 10mg f.wgt. of seed were surface-sterilized and conditioned (if required) as detailed in 2.1.1 above. Samples were ground in a perspex mortar and pestle on ice at a concentration of 6mg/100μL ice-cold grinding buffer (1mM EDTA, 10mM Tris, pH 8.0). For 1-dimensional SDS-PAGE, proteins were extracted under fully denaturing conditions (Laemmli, 1970). The sample was first diluted 1.5x with grind buffer before mixing the ground homogenate 1:1 with twice-strength (2x) Laemmli sample buffer followed by heating at 100°C for 5 min. Samples were centrifuged for 10 min at 12,600*g*

to remove any particulates before loading on to the gel.

For 2-dimensional electrophoresis samples to be stimulated with 3.4μM GR-24, 50mM ACC or 1μM thidiazuron were incubated in 9cm petri dishes on two circles of Whatman No.1 filter paper saturated with the test solution. Stimulation with ethylene took place in 8.87cm^3 gas-tight vials containing 1cm^3 s.H_2O; 250μL of head-space were removed and 250μL 0.1% ethylene were injected in through the silicone rubber/teflon seal to give a concentration of 32μL/L gas-phase. Ground samples were centrifuged, as above, for 10 min and an aliquot of the supernatant was extracted under denaturing conditions (O'Farrell, 1975) by mixing 1:1 with lysis buffer (9.5M urea, 2.0% (w/v) deionised Triton X-100, 5% (v/v) 2-mercaptoethanol, 2% ampholines (1.6% (w/v) pH 5-7, 0.4% (w/v) pH 3-10).

Molecular weight markers for SDS-PAGE (MW-SDS-70L, Sigma Chem. Co. LTD.) were bovine albumin (66,000), ovalbumin (45,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), carbonic anhydrase (29,000), bovine pancreas trypsinogen (24,000), soybean trypsin inhibitor (20,100) and bovine milk α-lactalbumin (14,200).

2.6.2 1-dimensional SDS-PAGE

Mini-gels (0.75mm thick) were run using the Bio-Rad Mini-Protean II system. The resolving gel was composed of 15% (w/v) acrylamide and 0.075% (w/v) bis-acrylamide in 0.375M Tris-HCl (pH 8.8), 0.1% (w/v) SDS and the stacking gel 4.75% (w/v) acrylamide and 0.25% (w/v) bis-acrylamide in 0.125M

Tris-HCl (pH 6.8), 0.1% (w/v) SDS (Laemmli, 1970; Casey, 1979). The gels were run at 200 V until the bromophenol blue dye front had reached the bottom of the gel.

2.6.3 Isoelectric focusing/2-dimensional PAGE

Capillary rod gels were run using the Bio-Rad mini-protean II 2-D insert. IEF and SDS-PAGE gels were prepared using PDA as opposed to bis-acrylamide as crosslinker. Use of PDA increases gel strength, overcoming the problems of manipulating small gels, and reduces background when using silver based protein stains (Heukeshoven and Dernick, 1985).

Isoelectric focusing was carried out under denaturing conditions as detailed by O'Farrell (1975) except for the inclusion of PDA. Gels were prepared containing 3.77% (w/v) acrylamide, 0.22% (w/v) PDA, 9.2M urea, 2% (w/v) deionised Triton X-100 and 2% ampholines (0.4% (w/v) pH 3-10 and 1.6% (w/v) pH 5-7). Equal volumes of extract were loaded at the basic end of the gels. No pre-focusing stages were included in the run and focusing was carried out at 500 V for 10 min followed by 750 V for at least 12 h.

After focusing each gel was extruded from the capillary tubes into $10cm^3$ equilibration buffer containing 62.5mM Tris pH 6.8, 2.3% (w/v) SDS, 10% (w/v) glycerol and stored immediately at -70°C until required. Gels to be used were quickly thawed and equilibrated according to Bies and Lazou (1990). Once thawed, 2-mercaptoethanol was added to 5% (v/v) and the gels were incubated at room temperature for 15 min. This solution was drained from the gels and replaced with equilibration buffer also containing 50mM iodoacetamide, for 20

min with one change of solution. Iodoacetamide was removed by washing the gels in two changes of equilibration buffer. This procedure removes 2-mercaptoethanol which cause the appearance of artifactual bands showing molecular weights of 50,000 to 65,000 in the second-dimension.

Second-dimension SDS-PAGE slab gels (1mm thick) were prepared as for 1-dimensional gels above, with two modifications. PDA replaced bis-acrylamide (1:1) as crosslinker and no stacking gel was used. Equilibrated gels were transferred to the top of the resolving gel in a small volume of running buffer. Molecular weight markers were diluted in molten 1% (w/v) agarose and cast in an IEF capillary tube. Once set, the gel was extruded and cut into 0.5cm lengths which gave acceptable intensities with silver stain. Running conditions were as for 1-dimensional SDS-PAGE (2.6.2).

All 2-dimensional PAGE gels were run in duplicate with no two identical samples being run in the second-dimension simultaneously.

2.6.4 Protein-staining of polyacrylamide gels

2.6.4.1 Coomassie staining

1-dimensional gels were stained for 30 min in 0.1% (w/v) Coomassie Brilliant Blue R-250 in 40% (v/v) methanol/10% (v/v) acetic acid followed by destaining in 30% (v/v) methanol/10% (v/v) acetic acid. Gels were preserved in 10% (v/v) glycerol.

2.6.4.2 Silver staining

2-dimensional gels were stained in an ammoniacal silver stain containing 0.1% (w/v) $AgNO_3$ as described by Hochstrasser *et al.*, (1988) except that all

incubation times were reduced by one-third.

2.6.5 Radiolabeling and Fluorography

Seed samples for labelling were surface-sterilized and conditioned, if necessary, as 2.1.1. Seeds, 10mg f.wgt., were washed onto 2.5cm Whatman No. 1 filter paper and transferred to 5cm polystyrene petri dishes containing 750μL s.H_2O containing 10μCi L-[^{35}S]methionine (1300 Ci/mM). Labelling was for 4 h at 33°C. Samples were then washed with s.H_2O and extracted as above for 2-D SDS-PAGE. 5μL aliquots of the extracted protein from each sample were mixed with 5μL of 10mg/cm^3 of bovine serum albumin and spotted onto 1.25cm Whatman GFA filter paper and precipitated by washing (1 x 30 min, 2 x 5 min) with ice-cold 10% (w/v) trichloroacetic acid, containing 10mM L-methionine to reduce background counts. A control filter, to which no sample was added, was included to give a background reading. The filters were then incubated for 5 min with 95% (v/v) ethanol, dried and placed in polypropylene scintillation vial inserts together with 5cm^3 scintillation fluid (Optiphase RIA., Pharmacia-LKB Biotechnology, Milton Keynes, U.K.). Counting was carried out for 5 min in an LKB Mini-Beta scintillation counter. The second dimension separation was carried out as detailed (2.6.3) except with ^{14}C-methylated protein markers (CFA.626 - Amersham International plc, Bucks., U.K.) replacing the non-radioactive markers. Markers were myosin (200,000), phosphorylase-b (92,500), bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000), lysozyme (14,300). The sample was run in duplicate though not simultaneously.

Gels were either silver stained as 2.5.4.2 or were prepared for fluorography essentially as described by Jen and Thatch (1982). Gels were first fixed in 30% (v/v) ethanol/10% (v/v) acetic acid for 1.5 h followed by incubation in 55% (v/v) acetic acid/30% (v/v) xylene/15% (v/v) ethanol containing 0.5% (w/v) 2,5-diphenyloxazole (PPO) for 1 h. The gel was then washed in s.H_2O for 1 h with one change of solution, to precipitate the PPO and removal the xylene, before drying overnight at 60°C between two sheets of cellophane in a Bio-Rad slab gel drier. Exposure to Fuji RX X-ray film took place at -70°C. Films were pre-flashed in order to improve sensitivity and give a linear relationship between spot intensity and radioisotope incorporation (Laskey and Mills, 1975). Films were developed and fixed using standard photographic procedures.

2.6.5 Laser scanning-densitometry

Densitometry was carried out using an LKB Ultroscan XL Enhanced Laser Densitometer. The vertical scanning increment along a track was set at 40μm. Three scans were made of each track, one through the centre and one 800μm to either side (i.e. a total scan width of 2400μm).

2.7 Protein assay

Soluble protein contents of samples were determined using the dye-binding assay of Bradford (1976) with γ-globulin as a standard. Soluble protein concentrations were determined using the samples prepared for 2-D electrophoresis; samples were diluted, as appropriate, with grinding buffer (1mM EDTA, 10mM Tris, pH 8.0) before analysis.

2.8 Measurement of respiration

Measurement of respiratory activity was performed using a Hansatech DW.2 Clark-type oxygen electrode. Calibration of the electrode took place at the start of each recording period. Zero oxygen was set at zero volts using sodium dithionite while the O_2 saturation point was determined using air saturated s.H_2O. Seed samples (5mg f.wgt. per replicate) were surface-sterilised and conditioned, if necessary, as detailed in 2.1.1 above, before transfer to the reaction chamber held at 33°C in the dark. Measurements made during the conditioning period were carried out in 750μL of air saturated s.H_2O for 1 h. When determining the effects of GR-24, ACC and thidiazuron the reaction volume was increased to 2.0cm^3. Initial respiration rates were determined in aerated s.H_2O for 30 min, the test solution was then added to the correct concentration and measurement continued for 1 h. Determination of the effect of ethylene was as for GR-24, ACC and thidiazuron except that after the initial 30 min in aerated s.H_2O, 500μL of 0.1% (v/v) ethylene was passed through the s.H_2O. At the end of the measuring period GR-24, ACC and thidiazuron stimulated samples were collected by filtration and incubated in fresh test solution at 33°C until the next measurement. Ethylene stimulated samples were collected by filtration and transferred to an 8.87cm^3 silicone rubber/teflon sealed borosilicate glass vial containing 1cm^3 s.H_{2O} through which was passed 250μL 0.1% (v/v) ethylene. At intervals over a 24 h period samples were returned to the reaction chamber containing the test solution (except at 24 h when measurements were made in s.H_2O) and measurement of oxygen activity was made for 1 h.

All determinations were made in triplicate. Respiration rates during

conditioning are plotted using 95% comparison intervals, while those during stimulation are plotted ± standard errors of the mean using the T-test for calculating the significance of the deviation from the zero-time (no stimulant) value (Sokal and Rohlf, 1981). Means are considered significantly different if $0.05 \geq P > 0.01$.

2.9 Sample preparation for Scanning Electron Microscopy

Samples were prepared essentially as Aber and Sallé (1983). Samples of seed were surface-sterilised and conditioned as detailed in 2.1.1 above. Stimulation by 1μM GR-24, 50mM ACC or 1μM thidiazuron took place in 9cm petri dishes in the dark at 33°C while stimulation by ethylene took place in 8.87cm^3 gas-tight vials. Samples of germinated seed were then washed and transferred to 2cm^3 eppendorfs to which was added 1.5 cm^3 4% (v/v) glutaraldehyde in 0.1M HEPES pH 7.4. The tissue was infiltrated by the fixative under vacuum for 20 min before gentle release of the vacuum and leaving the tubes to rotate end to end for approximately 8 weeks. The glutaraldehyde fixative was then carefully poured off and the samples washed several times in HEPES buffer before dehydration in a increasing ethanol series of 25, 35, 45, 60, 70, 85, 90, 95, 100, 100% (v/v). Samples were then critical point dried. Dried samples were mounted on SEM stubs using double-sided adhesive tape and coated with a thin layer of gold using a sputter-coater before examining in the SEM.

2.10 Amino acid analysis - HPLC

2.10.1 Sample preparation

Samples were surface sterilised and conditioned, if necessary, as 2.1.1 above. Samples were ground in an excess of methanol, transferred to $2cm^3$ eppendorfs and centrifuged at 12,600*g* for 5 min. The supernatant was removed and retained and the pellet further extracted with $1cm^3$ methanol at 4°C overnight. The samples were centrifuged (12,600*g*, 5 min) and the two supernatants combined and rotary-evaporated under vacuum. Extracts were resuspended in s.H_2O at a concentration of 100μL/30mg f.wgt. Comparisons between means for each amino acid, at 0 (n=3), 4 (n=2) and 7 days (n=2) were made using the T-test (Sokal and Rohlf, 1981).

2.10.2 HPLC apparatus and running parameters

The HPLC system (Kontron Instruments, Watford, Herts.) consisted of a 420 pump, 425 gradient former, 460 autosampler and injector, fitted after the injection loop with a stainless steel guard (2cm) and column (10 x 0.45cm) packed with Spherisorb 5μm ODS 2 (Phasesep Ltd., Clwyd, UK). Detection was by a Milton Roy Fluoro-Monitor 3 fluorescence detector with filters set for *o*-phthaldialdehyde amino acid derivatives. The column was maintained at 30°C by a model 480 oven controller.

Amino acids were analyzed as *o*-phthaldialdehyde derivatives on a C-18 column using a method adapted from Joseph and Marsden (1986). *o*-phthaldialdehyde stock reagent was prepared by dissolving 50mg in $1cm^3$ methanol and diluting to $7.5cm^3$ with 0.4M borate buffer, pH 9.5. Working

reagent was prepared by adding 10μL 2-mercaptoethanol to 1.5cm^3 *o*-phthaldialdehyde stock. All samples, standards, eluents and derivatizing reagents were passed through a 0.45μm filter prior to use. The gradient was produced using two eluents - A: 0.1M phosphate buffer (pH 8.3) with 2% (v/v) methanol and 2% (v/v) tetrahydrofluoran; B: 65% (v/v) methanol. Eluents were degassed with helium prior to use. The gradient was programmed as follows - 0 to 5 min, 20% to 35% B; 5 to 27 min., 35% to 100% B; 27 to 32 min, 100% B. 10μL sample was derivatized with 60μL working reagent. After 2 min 8μL derivatized sample was injected.

Samples were calibrated against standards containing 0.25nM of an amino acid standard mixture (Sigma Chem. Co. LTD.) with the addition of 0.25nM each of glutamine, γ-aminobutyric acid, serine and asparagine. All samples contained 0.25nM of homoserine as internal standard.

2.11 Glassware

All borosilicate glass vials, slab gel glass plates and the mortar and pestle used for protein sample preparation were soaked overnight in the detergent, RBS, then rinsed with tap water, followed by distilled water, before drying.

CHAPTER 3 - RESULTS

3.1 Germination studies

3.1.1 Conditioning

Obtaining *Striga hermonthica* seed separated from contaminating sand and maternal plant material in sufficient amounts for experimentation is very difficult. It was necessary, therefore, to use 3 different seed batches collected from Wad Medani, Sudan in 1981, 1987 and 1989. To determine optimum conditioning times for each *S. hermonthica* seed batch, germination percentages were measured in response to incubation in host root exudate after a conditioning period ranging from 24 h to 21 or 24 days.

The effect of duration of conditioning period on the germination of *S. hermonthica* seed collected from Wad Medani, Sudan in 1981, 1987 and 1989 is shown in Figs. 3.1-3.3. As shown by Figs 3.2 and 3.3, there is great variation in germination percentage after 48 h (G_{max}) both between experiments stimulated by the same host root exudate preparation but conditioned for different times (point sets A and B in Fig. 3.2; A, B and D in Fig. 3.3), and between experiments conditioned for the same time period before stimulation by different host root exudate preparations. As conditioning time increases variability between experiments stimulated by the same host root exudate preparation, but conditioned for different times, decreases (point sets E and F in Fig. 3.3). Seed collected in 1981 and 1989, conditioned for the respective period but incubated with s.H_2O instead of host root exudate failed to germinate.

Figure 3.1

Effect of length of the conditioning period on the germination of *Striga hermonthica* collected from Wad Medani, Sudan in 1987.

Seeds were conditioned for the periods indicated before germination for 48 h in host root exudate, ●; or distilled H_20, ○; (n=12).

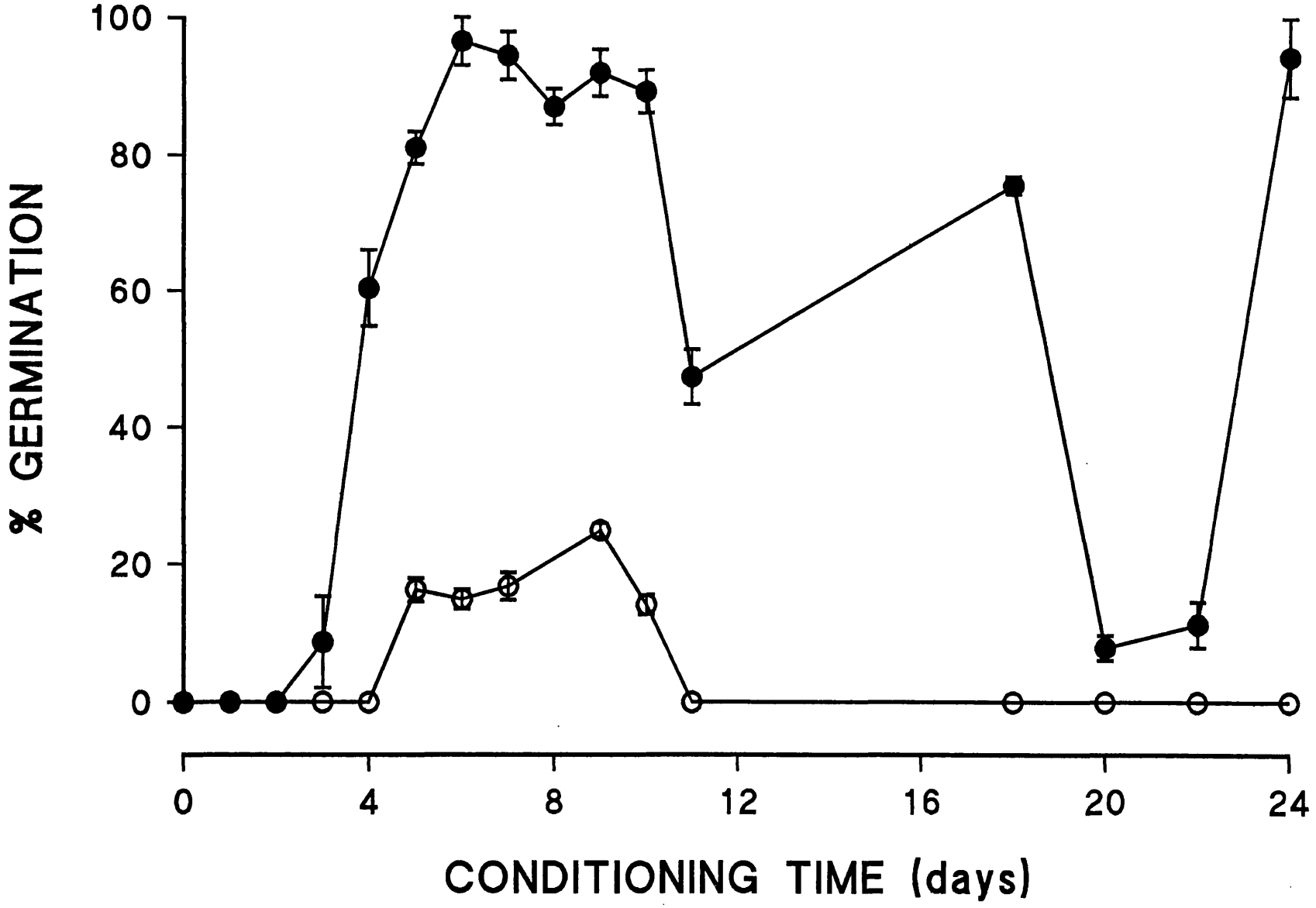

100
80
60
40
20
0
% GERMINATION
0
4
8
12
16
20
24
CONDITIONING TIME (days)

Figure 3.2

Effect of length of the conditioning period on the germination of *Striga hermonthica* collected from Wad Medani, Sudan in 1981.

Seeds were conditioned for the periods indicated before germination for 48 h in host root exudate; seeds incubated in s.H_2O did not germinate. Each data point is a separate experiment. (n=12).

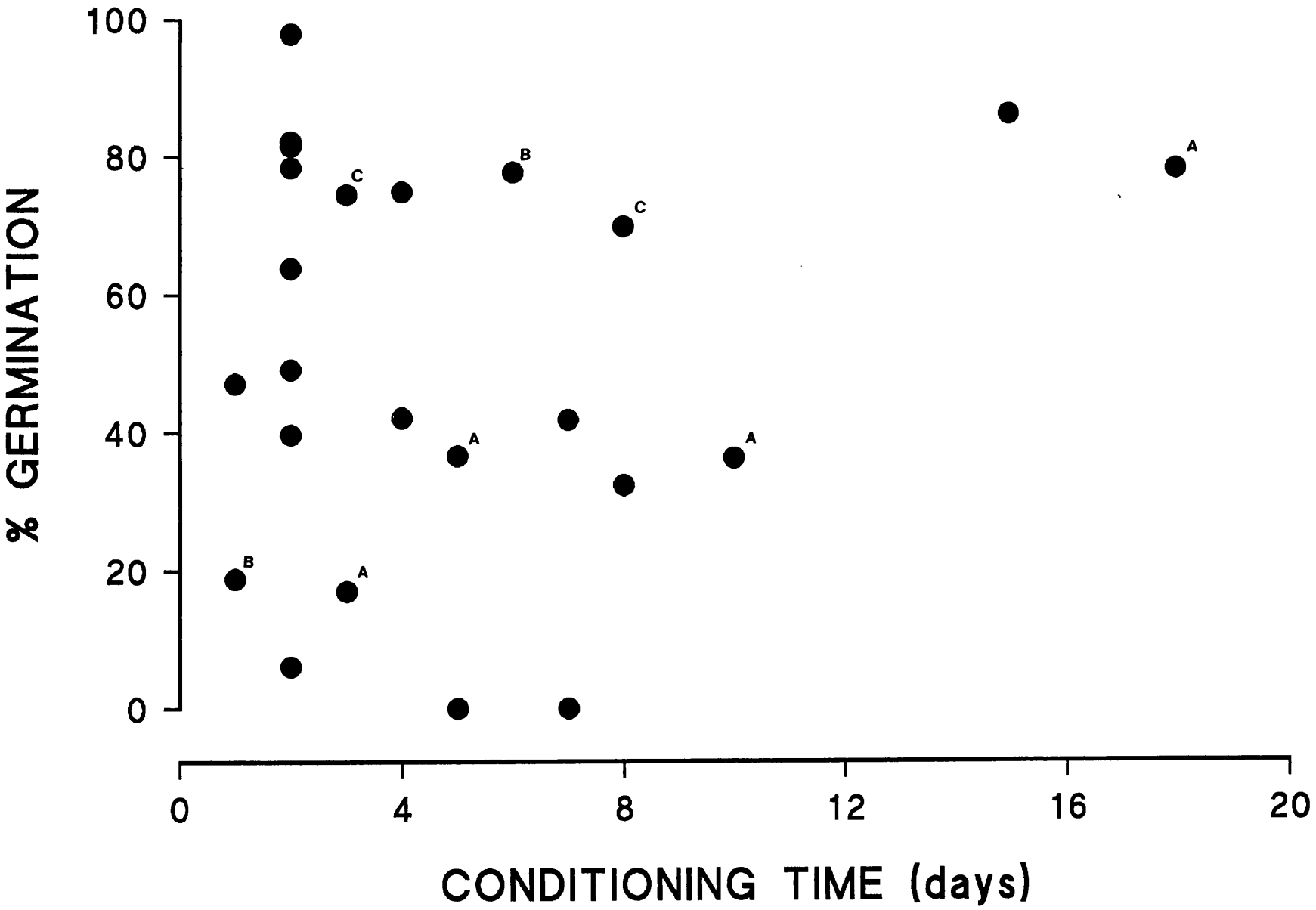
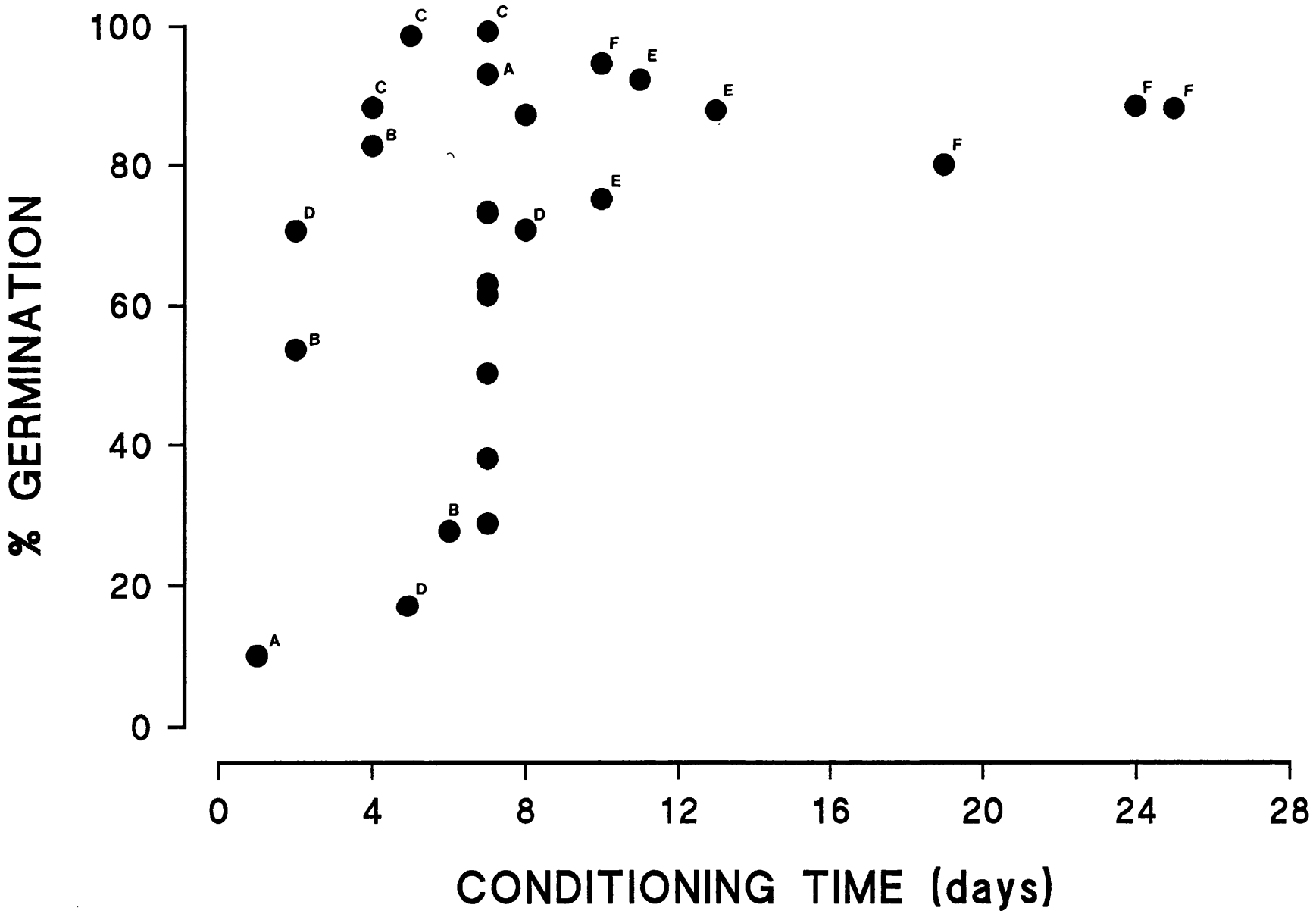

% GERMINATION
100
80
60
40
20
0
0
4
8
12
16
20
CONDITIONING TIME (days)
A
B
C
A
B
C
A
A

Figure 3.3

Effect of length of the conditioning period on the germination of *Striga hermonthica* collected from Wad Medani, Sudan in 1989.

Seeds were conditioned for the periods indicated before germination for 48 h in host root exudate; seeds incubated in s.H_2O did not germinate. Each data point is a separate experiment. (n=12).


% GERMINATION
100
80
60
40
20
0
0
4
8
12
16
20
24
28
CONDITIONING TIME (days)
A
B
C
D
E
F

In contrast to the data shown in Figs. 3.2 and 3.3, seed collected in 1987 and not surface-sterilised before conditioning did not germinate until 3 to 4 days after the initiation of imbibition (Fig. 3.1). In the case of seed collected in 1987, incubation in distilled H_2O resulted in a low percentage germination after 5 to 10 days conditioning. The difference in germination percentage following incubation in distilled H_2O, between the seed batches from 1981 or 1989 and that from 1987 may be due to the surface-sterilizing procedure. Seeds collected in 1981 and 1989 were washed in sodium hypochlorite prior to conditioning while seeds collected in 1987 were not (see 2.1.1). Some spontaneous germination usually occurs during the conditioning process, it is possible that treatment with sodium hypochlorite stimulates those seeds which are likely to germinate during conditioning, to germinate earlier during the conditioning process (see 1.1.5).

Conditioning times were set at 2 days for seed from 1981, 8 days for 1987 and 7 days for 1989 seed.

3.1.2 Conditioned seed - 7 days imbibition

3.1.2.1 Stimulation by host root exudate

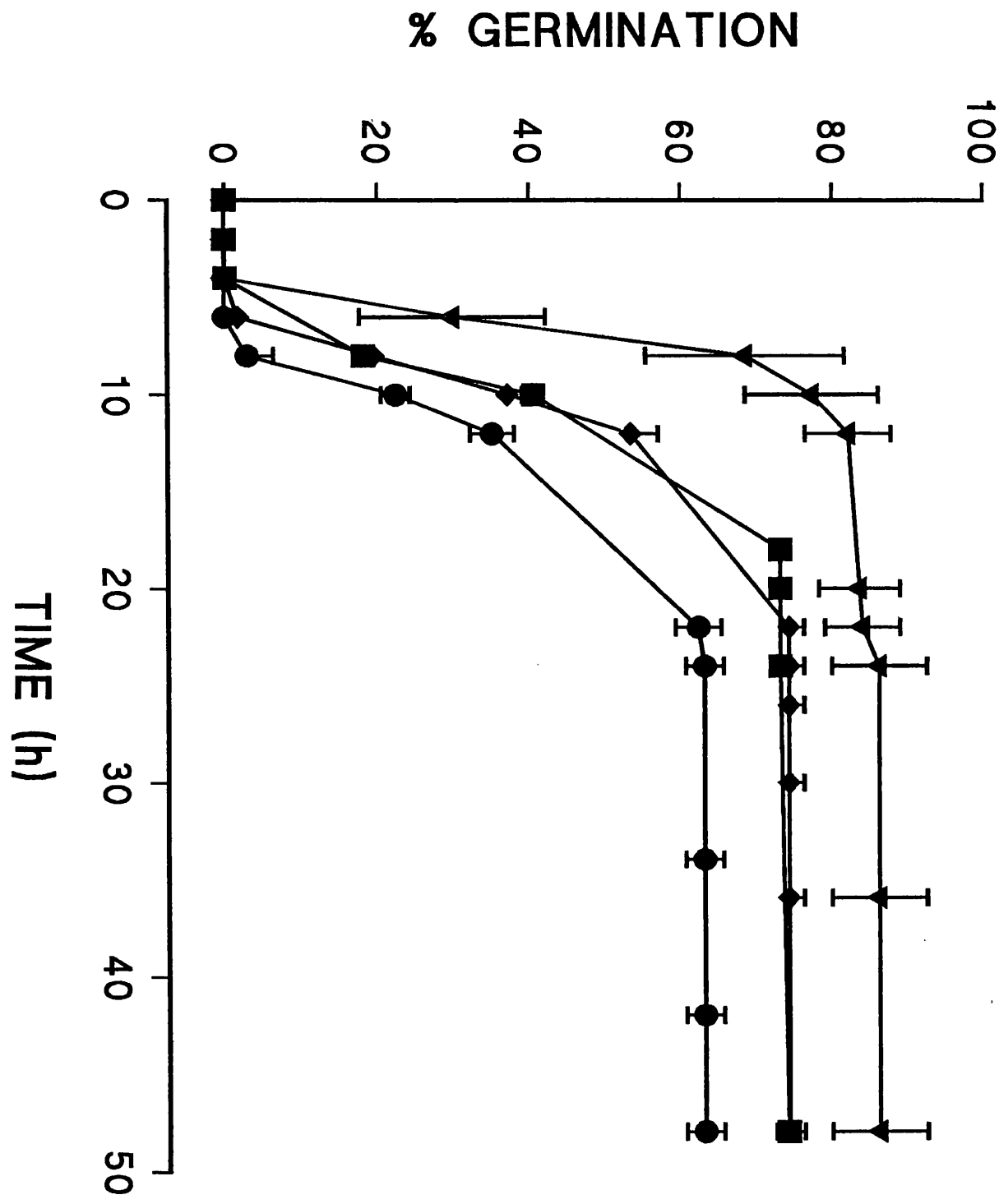
After 7 days conditioning, seeds of *S. hermonthica* germinate rapidly following application of host root exudate with protrusion of the radicle visible between 6 and 8 h (Fig. 3.4). As mentioned in 3.1.1 above, maximum germination varied substantially, with an average maximum germination percentage after 48 h (G_{max}) for the 4 replicates of 74.2 ± 9.4

The increase in number of seeds germinating, starting from a germination value of ≤ 5% and thus not including any lag-time, was linear over

Figure 3.4

Effect of host root exudate on the germination of *Striga hermonthica.*

Seeds were conditioned for 7 days before transfer to host root exudate at time zero (± S.D., n=12). Each curve (●, ■, ▼, ◆) represents a separate experiment. Seed incubated in s.H_2O instead of host root exudate did not germinate.


% GERMINATION
0
20
40
60
80
100
TIME (h)
0
10
20
30
40
50

the first 4 h irrespective of incubation medium and therefore initial germination rates (R_i) were calculated by linear regression (Table 3.1). Maximum percentage germination is reached by, at most, 24h in all experiments with those of low tG_{50} (time to 50% G_{max}) attaining near maximal germination faster. Calculation of a value for tG_{50} gives a better indication of the strength of the response to host root exudate than does R_i since the tG_{50} takes into account any lag-time. Seeds stimulated with host root exudate had a morphology as shown in Figs. 3.5A. This morphology is considered to be normal, with elongation of the radicle and the presence of root hairs, predominantly at the radicle tip.

3.1.2.1.1 Inhibition by AVG

To determine whether stimulation of germination in *S. hermonthica* by *Sorghum* host root exudate, involves the production of ethylene, seeds were incubated with host root exudate containing 1-1000μM of the ethylene biosynthesis inhibitor AVG. Incubation with AVG results in a reduction of G_{max} in a concentration dependent manner (Fig. 3.8 and Table 3.2). Increasing the concentration of AVG results in little change to the tG_{50} while causing a great reduction in R_i (Table 3.2). Plotting the response of *S. hermonthica* to inhibitor concentration results in an average I_{50} (inhibitor concentration causing 50% reduction in G_{max}) of 24μM (Fig. 3.9A,B). Seeds which germinated in the presence of AVG had a morphology indistinguishable from those which germinated in host root exudate alone.

Germination inhibition by AVG can be overridden by application of the product of the inhibited reaction, ACC (Fig. 3.10). Addition of ACC to a final concentration of 50mM increases the G_{max} to the same maximum irrespective of

Table 3.1

Germination indices for host root exudate stimulated germination. Data from Figure 3.4.

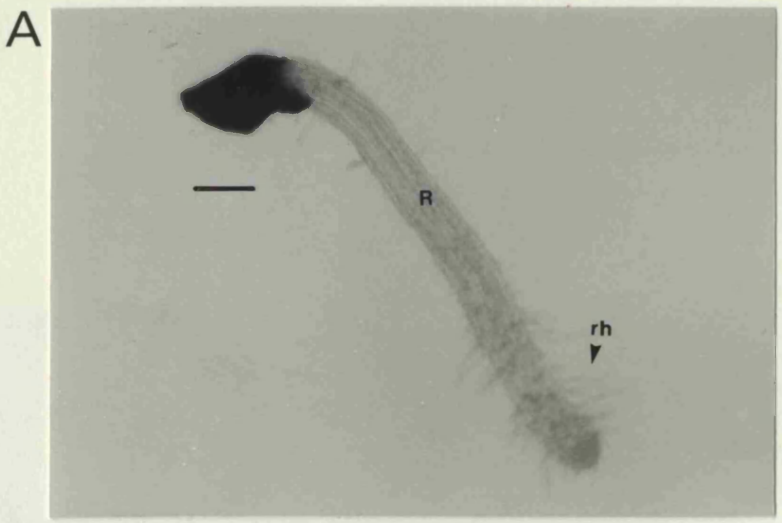
Exp.	R_i	tG_{50}	G_{max}
I	17.12	7	86
II	6.45	9	74
III	8.03	11	63
IV	8.82	9	74
$\bar{X}$	10.1	9	74.2

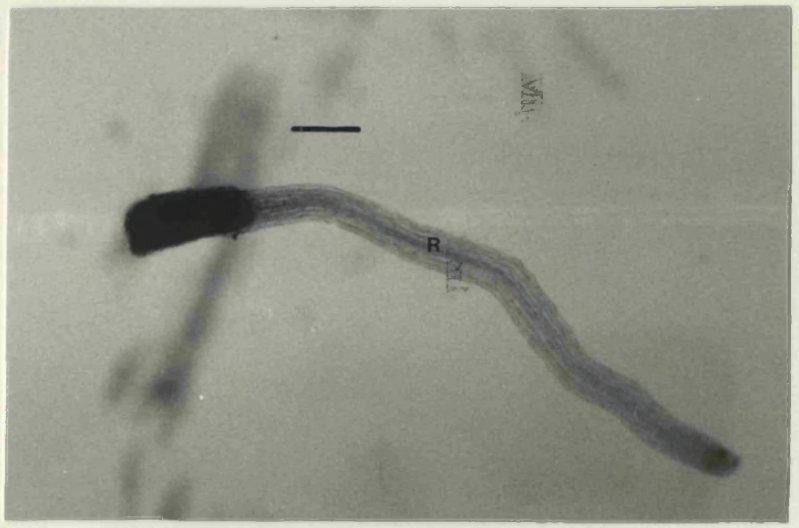
G_{max} - germination after 48h; R_i - initial germination rate; tG_{50} - time to 50% of G_{max}.

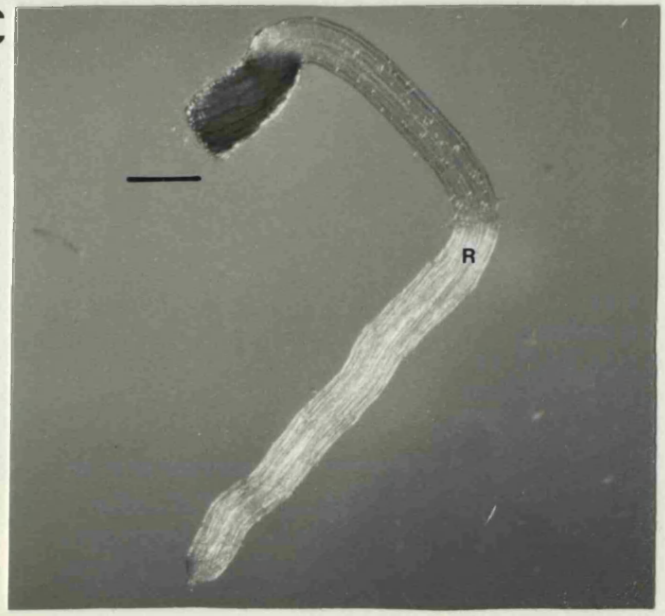
Figure 3.5

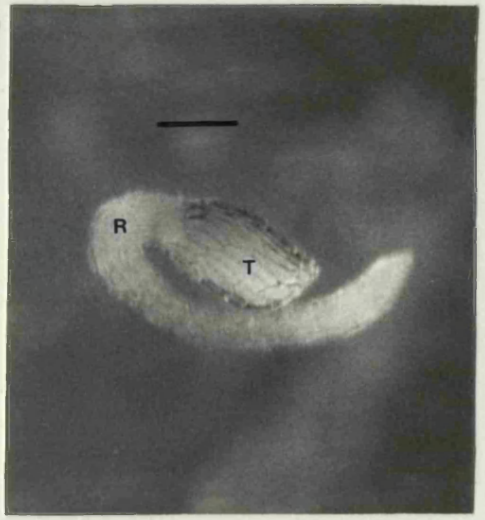
Light photomicrograph of *Striga hermonthica* seed conditioned for 24 h (**F**) or 7 days (**A, B, C, D, E, G**) before incubation in: **A.** host root exudate, 24 h; **B.** ethylene, 24 h; **C.** 3.4μM GR-24, 24 h; **D.** 3.4μM GR-24, 10 h; **E.** 50mM ACC, 24 h; **F.** 50mM ACC + 1mM $CoCl_2$, 40 h; **G.** 1μM thidiazuron, 24 h. Bar = 200μm.

c = cotyledons, R = radicle, rh = root hairs, T = testa.

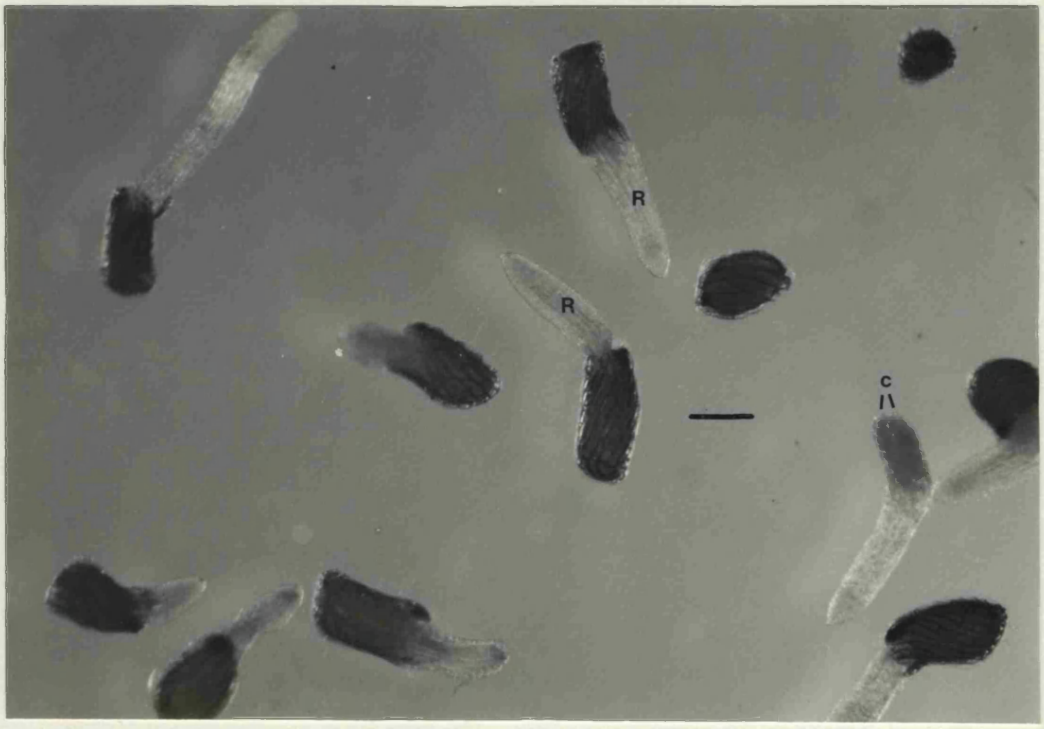

A
R
rh


B
R


C
R


D
R
T

E



F

G

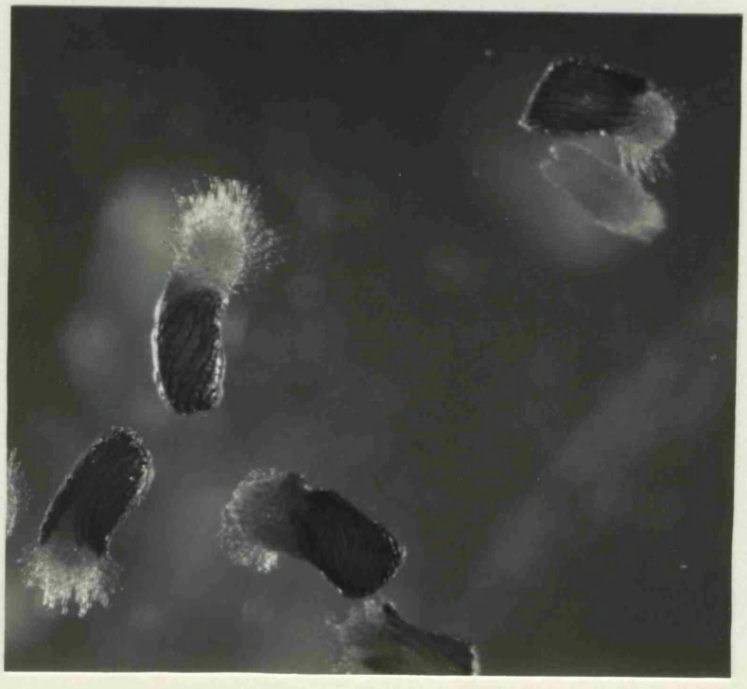


Figure 3.6

SEM photomicrographs of *Striga hermonthica* seed conditioned for 7 days before incubation in: **A** and **B** - 3.4μM GR-24, 24 h; **C** and **D** - 50mM ACC, 24 h. Bar = 100μm.

t = testa, r = radicle.

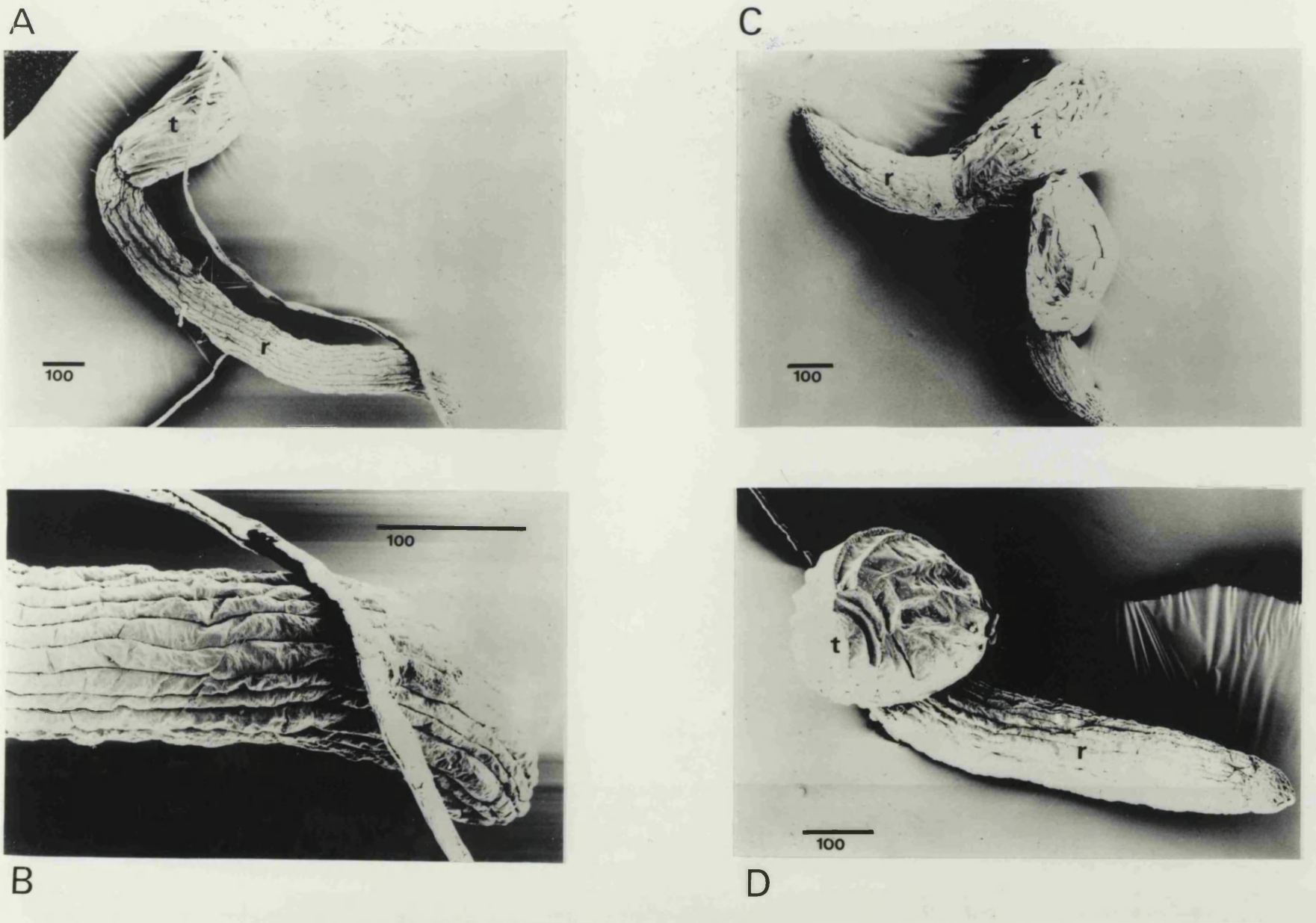
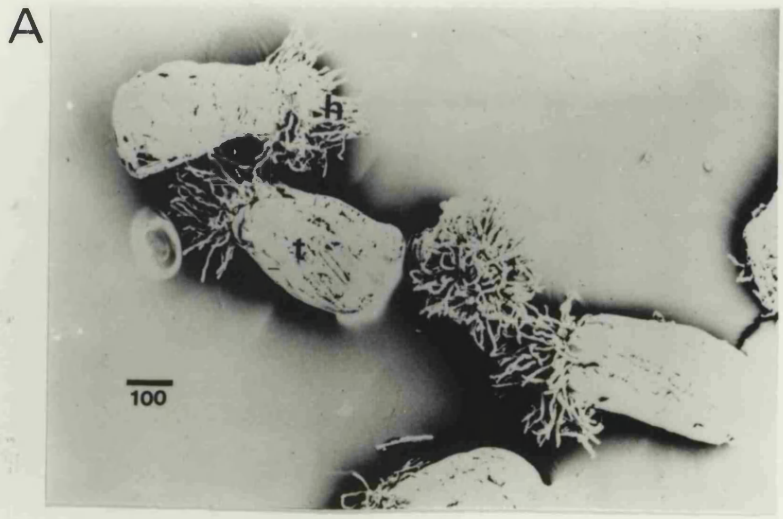
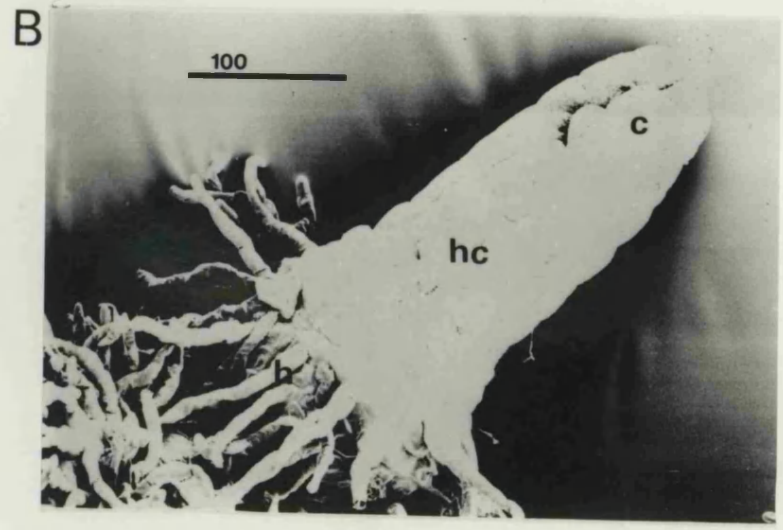

A
t
r
100
C
t
r
100
100
B
t
r
100
D

Figure 3.7

SEM photomicrographs of *Striga hermonthica* seed conditioned for 7 days before incubation in 1μM thidiazuron. Bar = 100μm.

c = cotyledon, hc = hypocotyl, h = root hairs, t = testa.


A
h
t
100


B
100
c
hc
h

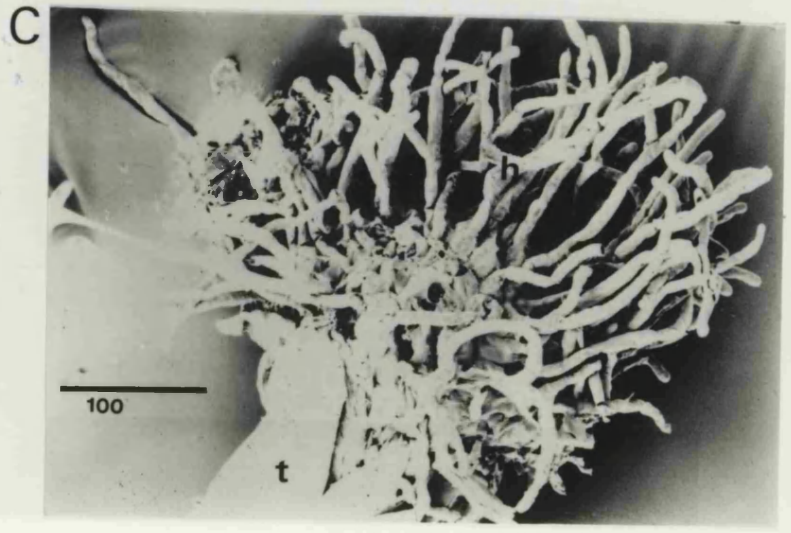
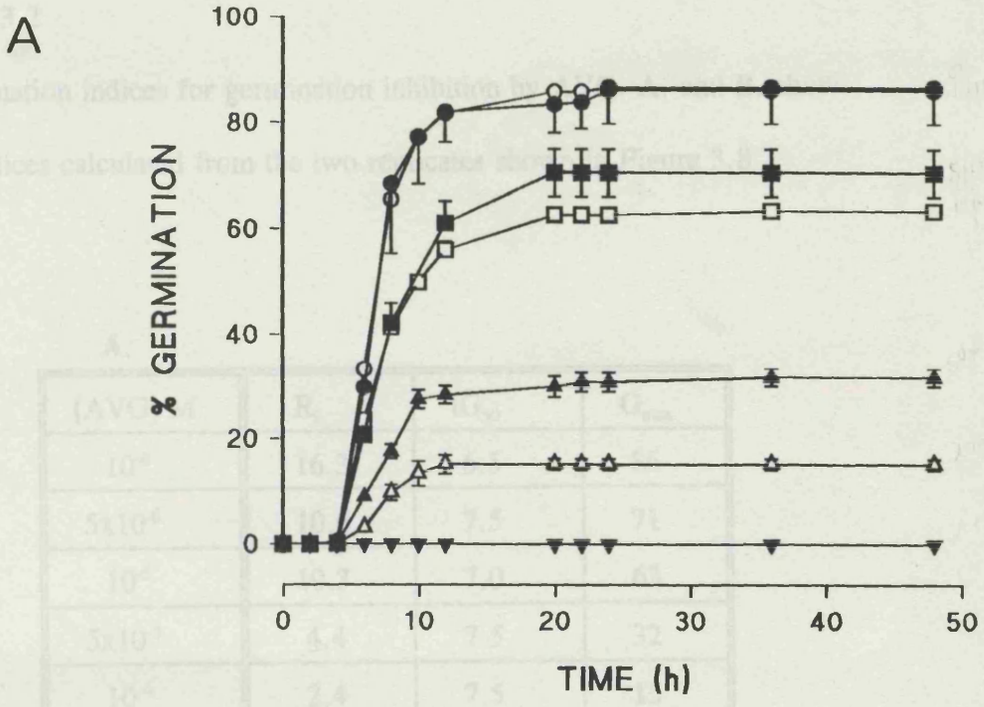

C
h
100
t

Figure 3.8

Effect of AVG on *Striga hermonthica* germination stimulated by host root exudate.

Seeds were conditioned for 7 days before incubation in host root exudate containing different concentrations of AVG: ●, OμM; ○, 1μM; ■, 5μM; □, 10μM; ▲, 50μM; ▵, 100 μM; ▼, 1 mM. Error bars indicate ± S.D. (n=12). Each graph shows a separate experiment. Seed incubated in s.H_2O instead of host root exudate did not germinate.


A
100
80
60
40
20
0
% GERMINATION
0
10
20
30
40
50
TIME (h)

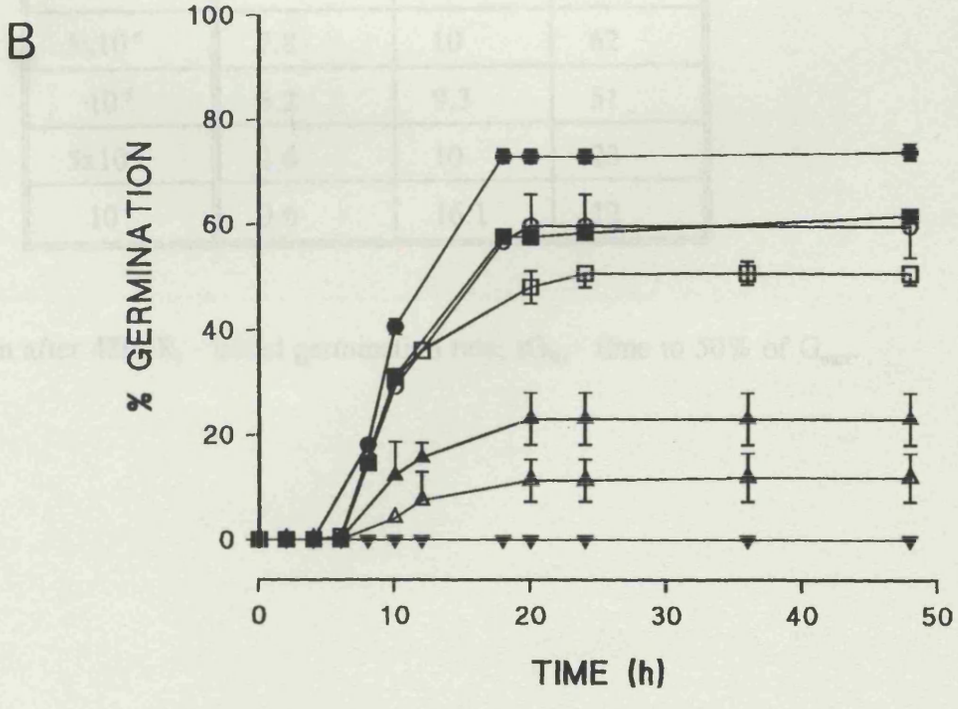

B
100
80
60
40
20
0
% GERMINATION
0
10
20
30
40
50
TIME (h)

Table 3.2

Germination indices for germination inhibition by AVG. **A.** and **B.** show the indices calculated from the two replicates shown in Figure 3.8.

A.

[AVG] M	R_i	tG_{50}	G_{max}
10^{-6}	16.3	6.5	86
$5x10^{-6}$	10.6	7.5	71
10^{-5}	10.3	7.0	63
$5x10^{-5}$	4.4	7.5	32
10^{-4}	2.4	7.5	15

B.

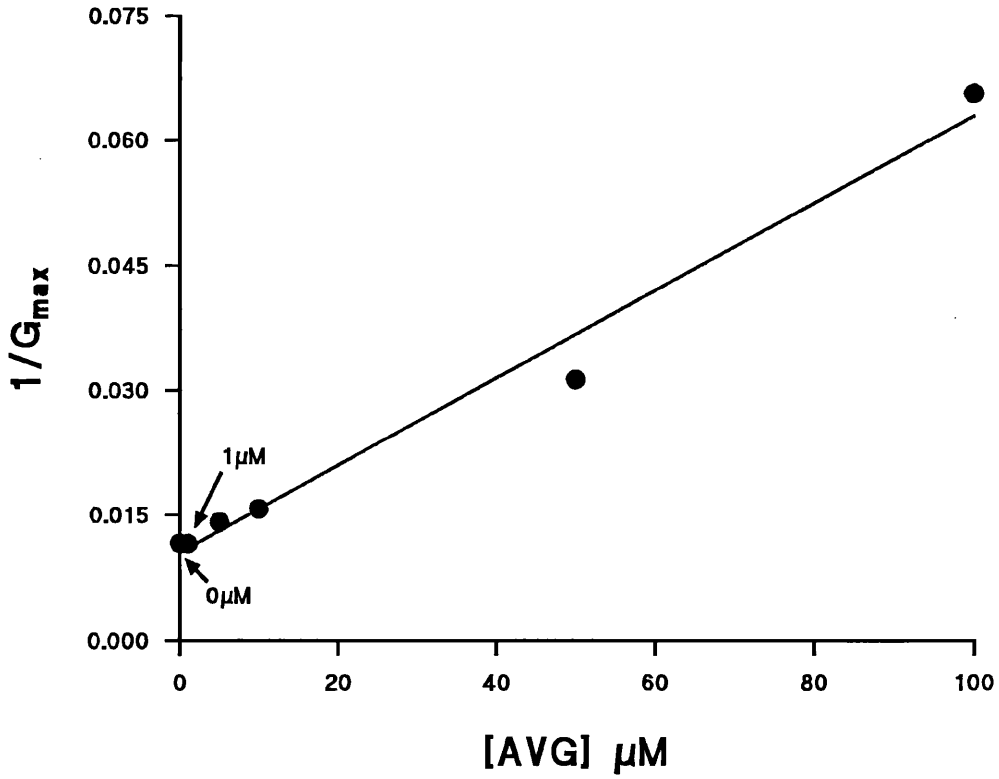
[AVG] M	R_i	tG_{50}	G_{max}
10^{-6}	7.3	10	60
$5x10^{-6}$	7.8	10	62
10^{-5}	6.2	9.3	51
$5x10^{-5}$	2.6	10	23
10^{-4}	0.6	16.1	12

G_{max} - germination after 48h; R_i - initial germination rate; tG_{50} - time to 50% of G_{max}.

Figure 3.9

Reciprocal germination plots of AVG inhibition of host root exudate stimulated germination. Each graph represents a separate experiment. Data from Figure 3.8.

A


0.075
0.060
0.045
0.030
0.015
0.000
1/Gmax
1μM
0μM
0
20
40
60
80
100
[AVG] μM

B

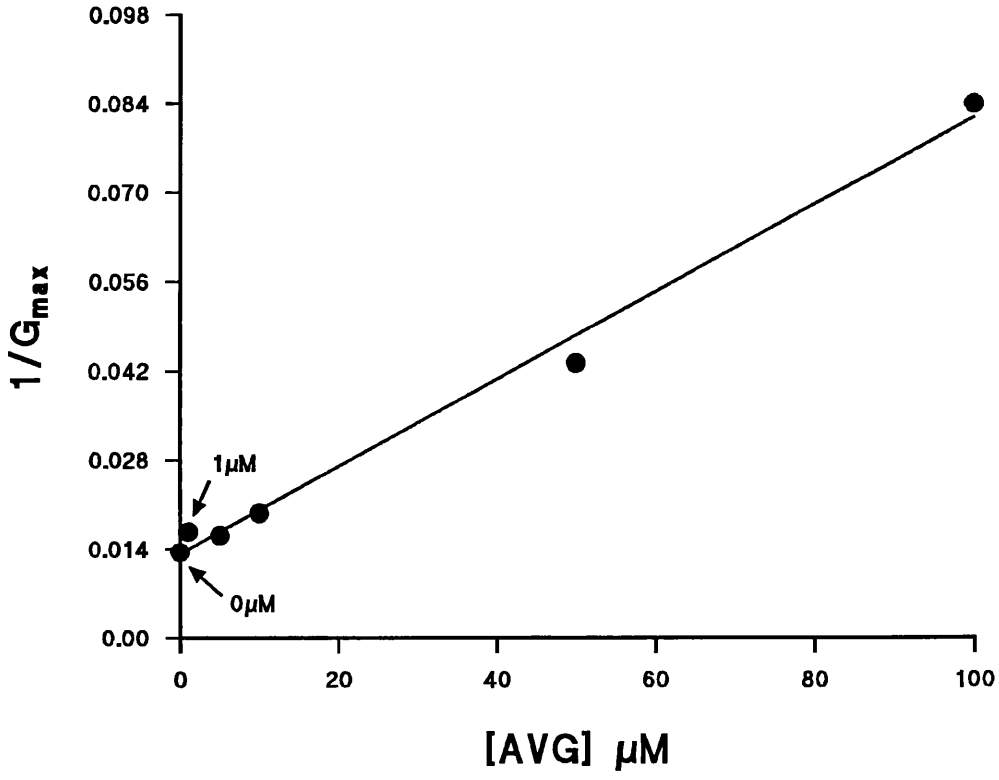
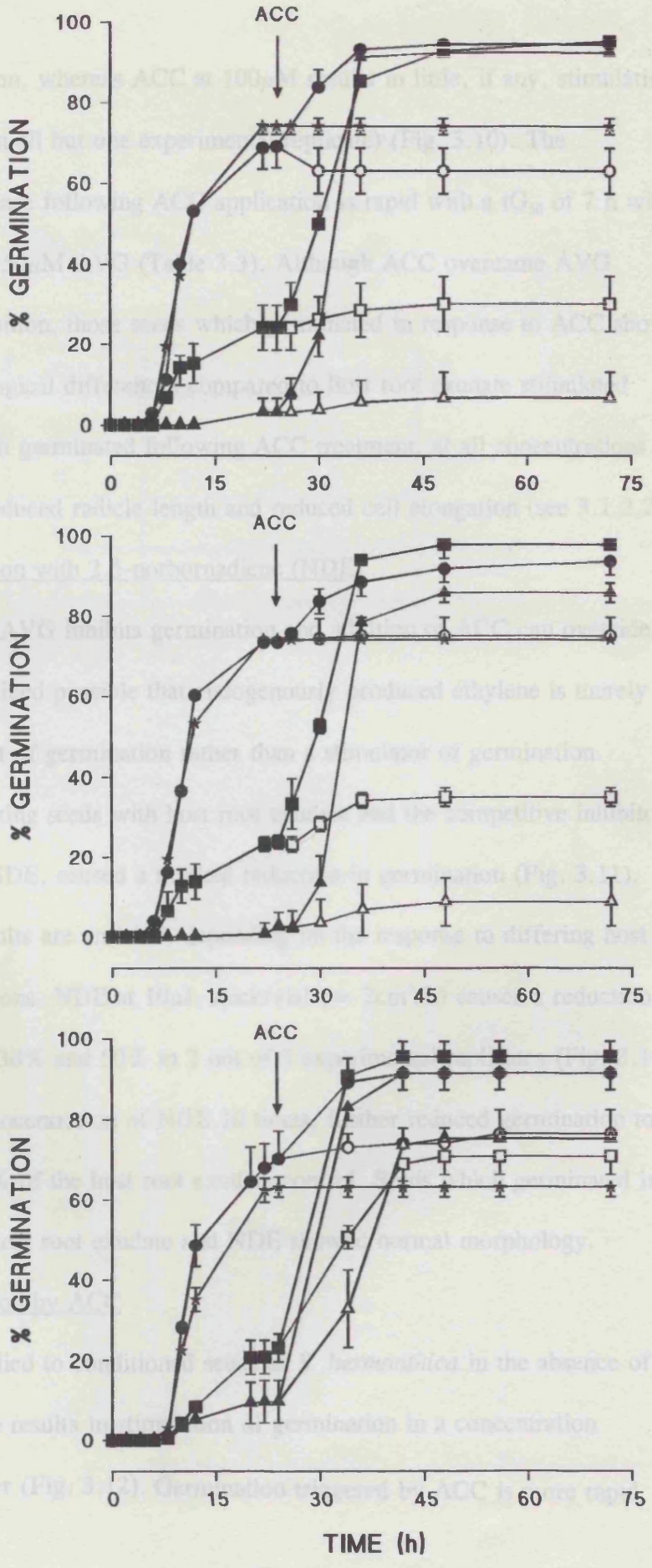

0.098
0.084
0.070
0.056
0.042
0.028
0.014
0.00
1/Gmax
1μM
0μM
0
20
40
60
80
100
[AVG] μM

Figure 3.10

Overriding AVG germination inhibition by addition of ACC.

Seeds were conditioned for 7 days before incubation for 24 h in host root exudate containing either 0.1μM AVG, ●; 50μM AVG, ■; or 1mM AVG, ▲. ACC was then added to give 50mM (closed symbols) or 100μM (open symbols). Control treatment, ★, was of host root exudate only. Error bars indicate ± S.D. (n=12). Each graph shows a separate experiment. Seed incubated in s.H_2O instead of host root exudate did not germinate.


ACC
% GERMINATION
100
80
60
40
20
0
0
15
30
45
60
75
TIME (h)

AVG concentration, whereas ACC at 100μM results in little, if any, stimulation of germination (in all but one experimental replicate) (Fig. 3.10). The germination increase following ACC application is rapid with a tG_{50} of 7 h with 50mM ACC and 50μM AVG (Table 3.3). Although ACC overcame AVG germination inhibition, those seeds which germinated in response to ACC showed striking morphological differences compared to host root exudate stimulated seeds. Seed which germinated following ACC treatment, at all concentrations tested, showed reduced radicle length and reduced cell elongation (see 3.1.2.2).

3.1.2.1.2 Inhibition with 2,5-norbornadiene (NDE)

Although AVG inhibits germination and addition of ACC can override this inhibition it remained possible that endogenously produced ethylene is merely a necessary product of germination rather than a stimulator of germination. However, incubating seeds with host root exudate and the competitive inhibitor of ethylene action NDE, caused a marked reduction in germination (Fig. 3.11). Although the results are variable, depending on the response to differing host root exudate preparations, NDE at 10μL stock/vial ($\approx$ 2cm^3/L) causes a reduction in G_{max} of between 30% and 50% in 2 out of 3 experimental replicates (Fig. 3.11). Increasing the concentration of NDE 10 times, further reduced germination to a maximum of 17% of the host root exudate control. Seeds which germinated in the presence of host root exudate and NDE showed normal morphology.

3.1.2.2 Stimulation by ACC

ACC applied to conditioned seeds of *S. hermonthica* in the absence of host root exudate results in stimulation of germination in a concentration dependent manner (Fig. 3.12). Germination triggered by ACC is more rapid

Table 3.3

tG_{50} for 50mM ACC stimulation of AVG germination inhibition. Data from Figure 3.10.

	[AVG]	
Exp.	50μM	1mM
I	5.5	5.5
II	7.0	8.1
III	7.0	8.7
$\bar{X}$	6.5	7.4

tG_{50} - time to 50% of G_{max}.

Figure 3.11

Effect of NDE on *Striga hermonthica* germination stimulated by host root exudate.

Seeds were conditioned for 7 days before incubation in host root exudate with NDE added at two concentrations as described in 2.1.1: ■, 10μL/vial; □, 100μL/vial. Control treatment, ●, was of host root exudate. Error bars indicate ± S.D. (n=12). Seed incubated in s.H_2O instead of host root exudate did not germinate. Each graph shows a separate experiment.

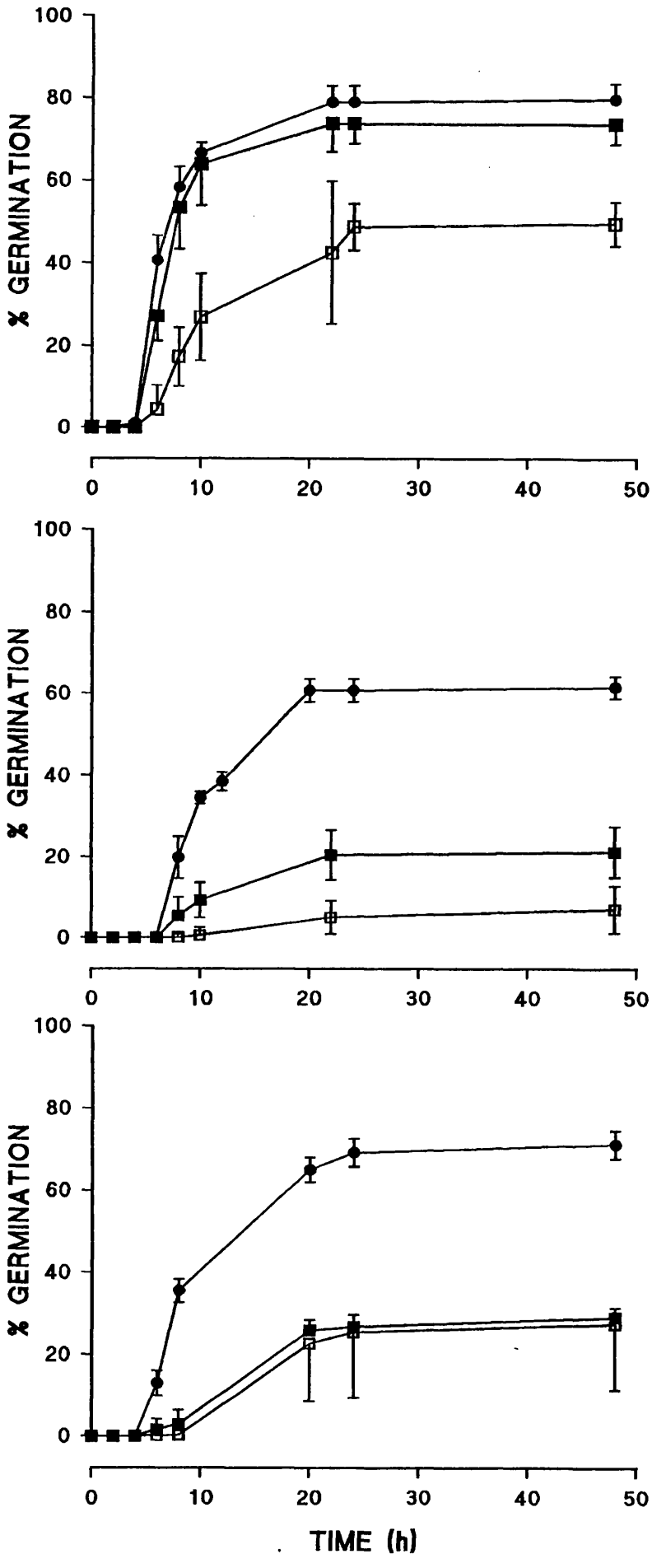
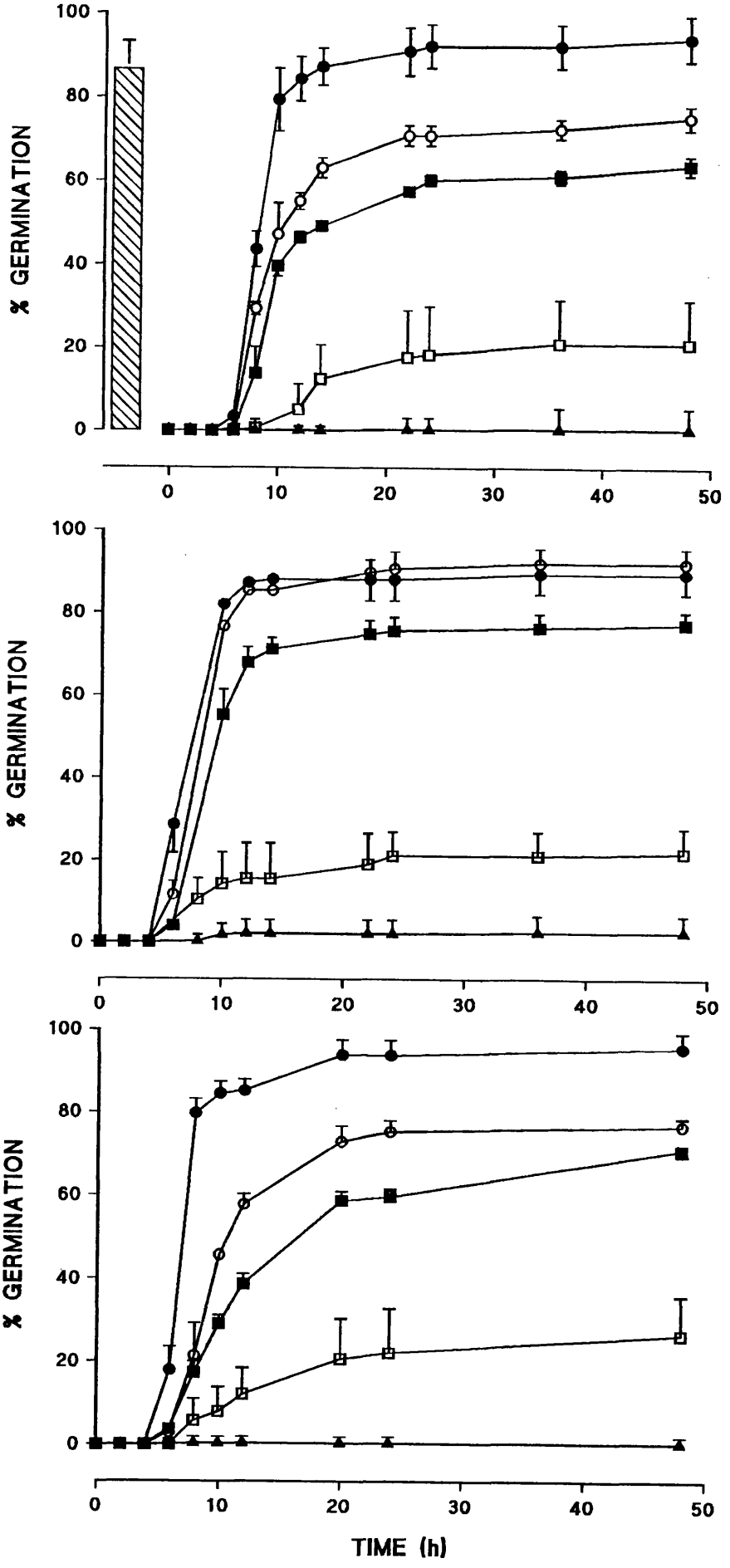

% GERMINATION
100
80
60
40
20
0
0
10
20
30
40
50
% GERMINATION
100
80
60
40
20
0
0
10
20
30
40
50
% GERMINATION
100
80
60
40
20
0
0
10
20
30
40
50
TIME (h)

Figure 3.12

Effect of ACC on the germination of 7 day conditioned *Striga hermonthica*.

Seeds, conditioned for 7 days, were incubated at time zero with ●, 50mM; ○, 10mM; ■, 5mM; □, 1mM or▲, 100μM ACC. Hatched bar represents germination with host root exudate at 48 h. Error bars indicate ± S.D. (n=12). Each graph shows a separate experiment.


% GERMINATION
100
80
60
40
20
0
0
10
20
30
40
50
% GERMINATION
100
80
60
40
20
0
0
10
20
30
40
50
% GERMINATION
100
80
60
40
20
0
0
10
20
30
40
50
TIME (h)

than that stimulated by host root exudate, shown by higher R_i and lower tG_{50} values (Table 3.4). Incubation with ACC at all concentrations tested resulted in atypical germination - the radicles were greatly reduced in length with a reduction in cell elongation and the absence of root hairs (Figs. 3.5E, 3.6C and D).

Incubation of conditioned seeds with ACC at 50mM and $CoCl_2$, an inhibitor of ACC oxidase (see Fig. 1.2), at 50mM, 10mM and 1mM results in inhibition of germination proportional to the cobalt concentration (Fig. 3.13). The lag-time is generally increased and G_{max} decreased with increasing inhibitor concentration (Fig. 3.13).

Some of the experiments detailed in 3.1.2.1.1 and 3.1.2.2 were initially performed with *S. hermonthica* seed collected in 1981 and 1987 and gave a similar pattern of results (Figs. 3.14-3.16). Although the degree of stimulation by host root exudate differs between the replicates of seed from 1981 (see 3.1.1 above) incubation with AVG substantially reduces G_{max} in both cases. Treatment with 10mM ACC in the absence of host root exudate results in stimulation of germination (Fig. 3.14A and B) although the response to ACC is low compared to that observed for seed from 1989 (*c.f.* Fig. 3.12). Germination of seed collected in 1987 is inhibited by AVG in a similar manner as the 1989 seed, being completely inhibited by 1mM while 1μM has no effect (Fig. 3.15). As with 1989 seed, addition of ACC to seed inhibited by AVG results in a concentration dependent increase in percentage germination (Fig 3.16).

Table 3.4

Germination indices for 50mM ACC stimulated germination. Data from Figure 3.12.

Exp.	R_i	tG_{50}	G_{max}
I	19.0	8.2	94.2
II	13.6	7.1	89.4
III	19.9	6.8	95.6
$\bar{X}$	17.5	7.4	93.1

G_{max} - germination after 48h; R_i - initial germination rate; tG_{50} - time to 50% of G_{max}.

Figure 3.13

Inhibition of ACC stimulated germination of 7 day conditioned *Striga hermonthica* seed by $CoCl_2$.

Seeds were incubated in 50mM ACC containing different concentrations of $CoCl_2$: ■, 50mM; ○, 10mM; ●, 1mM. Error bars indicate ± S.D. (n=12). Each graph shows a separate experiment.

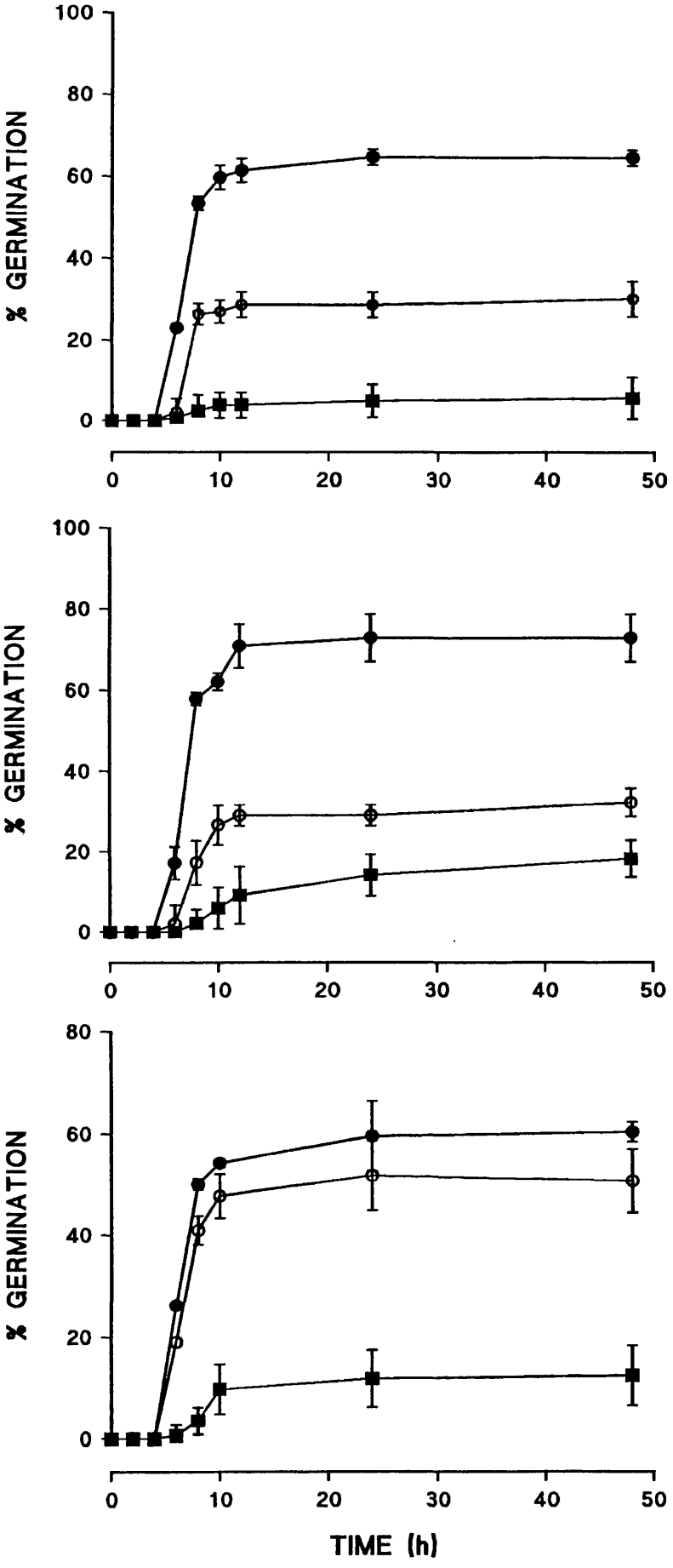
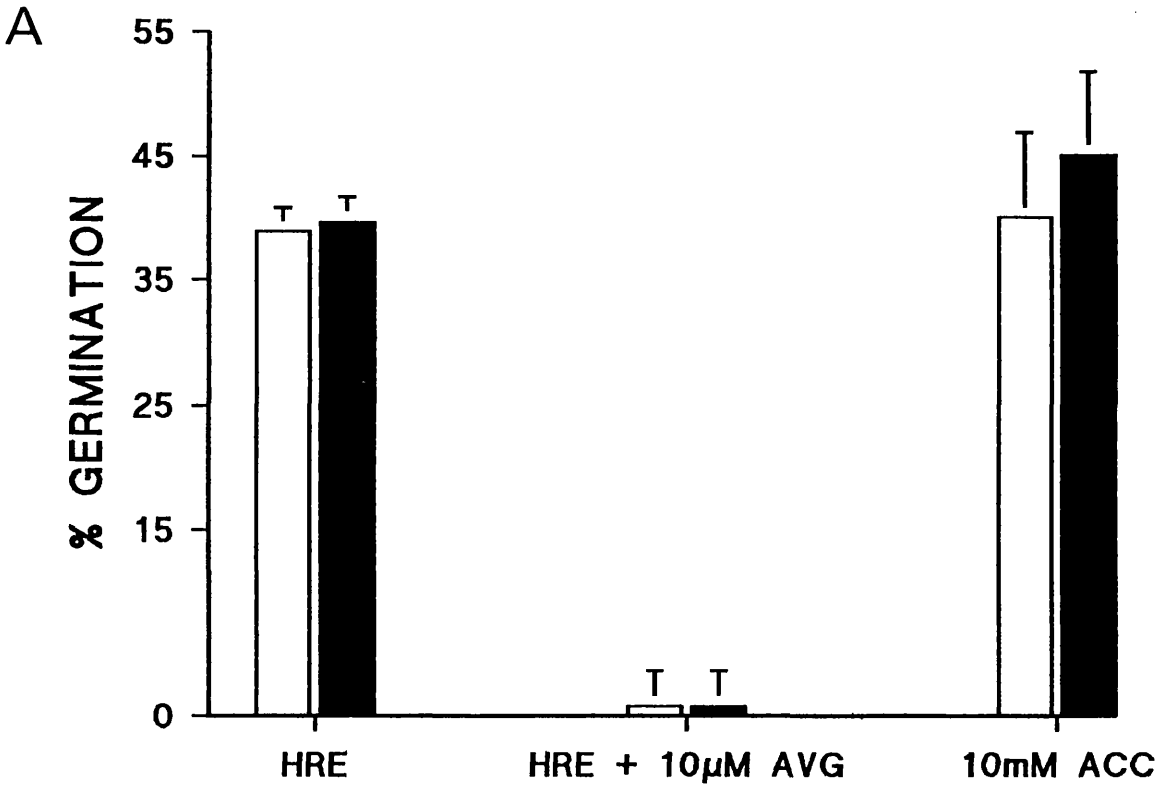

% GERMINATION
100
80
60
40
20
0
0
10
20
30
40
50
% GERMINATION
100
80
60
40
20
0
0
10
20
30
40
50
% GERMINATION
80
60
40
20
0
0
10
20
30
40
50
TIME (h)

Figure 3.14

Effect of AVG or ACC on the germination of *Striga hermonthica* seed collected in Wad Medani, Sudan in 1981.

Seeds, conditioned for 2 days were incubated in either host root exudate, host root exudate and 10μM AVG, 10mM ACC (replicate A) or 1μM, 0.1mM and 10mM ACC (replicate B). A and B show the results of separate experiments. Germination was measured after 24 h, open bars; or 48 h, closed bars. Error bars indicate S.D. (n=12). Seed incubated in distilled H_2O instead of host root exudate did not germinate.


A
55
45
35
25
15
0
% GERMINATION
HRE
HRE + 10μM AVG
10mM ACC

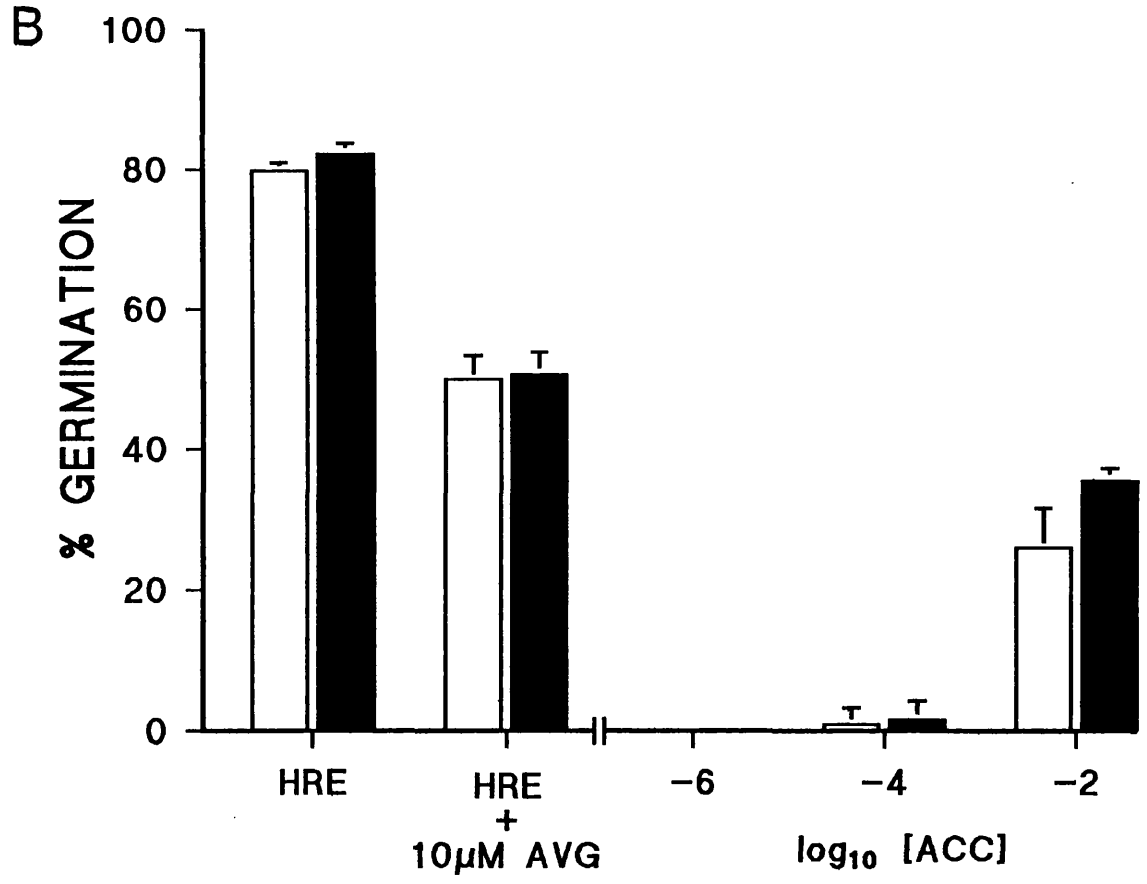

B
100
80
60
40
20
0
% GERMINATION
HRE
HRE + 10μM AVG
-6
-4
-2
log10 [ACC]

Figure 3.15

Effect of AVG on host root exudate stimulated germination of *Striga hermonthica* seed collected in Wad Medani, Sudan in 1987.

Seeds were conditioned for 8 days before incubation in host root exudate (HRE) or host root exudate containing 0.1μM to 1mM AVG. Germination was measured at 48 h. Error bars indicate S.D. (n=9). Seed incubated in distilled H_2O instead of host root exudate did not germinate.

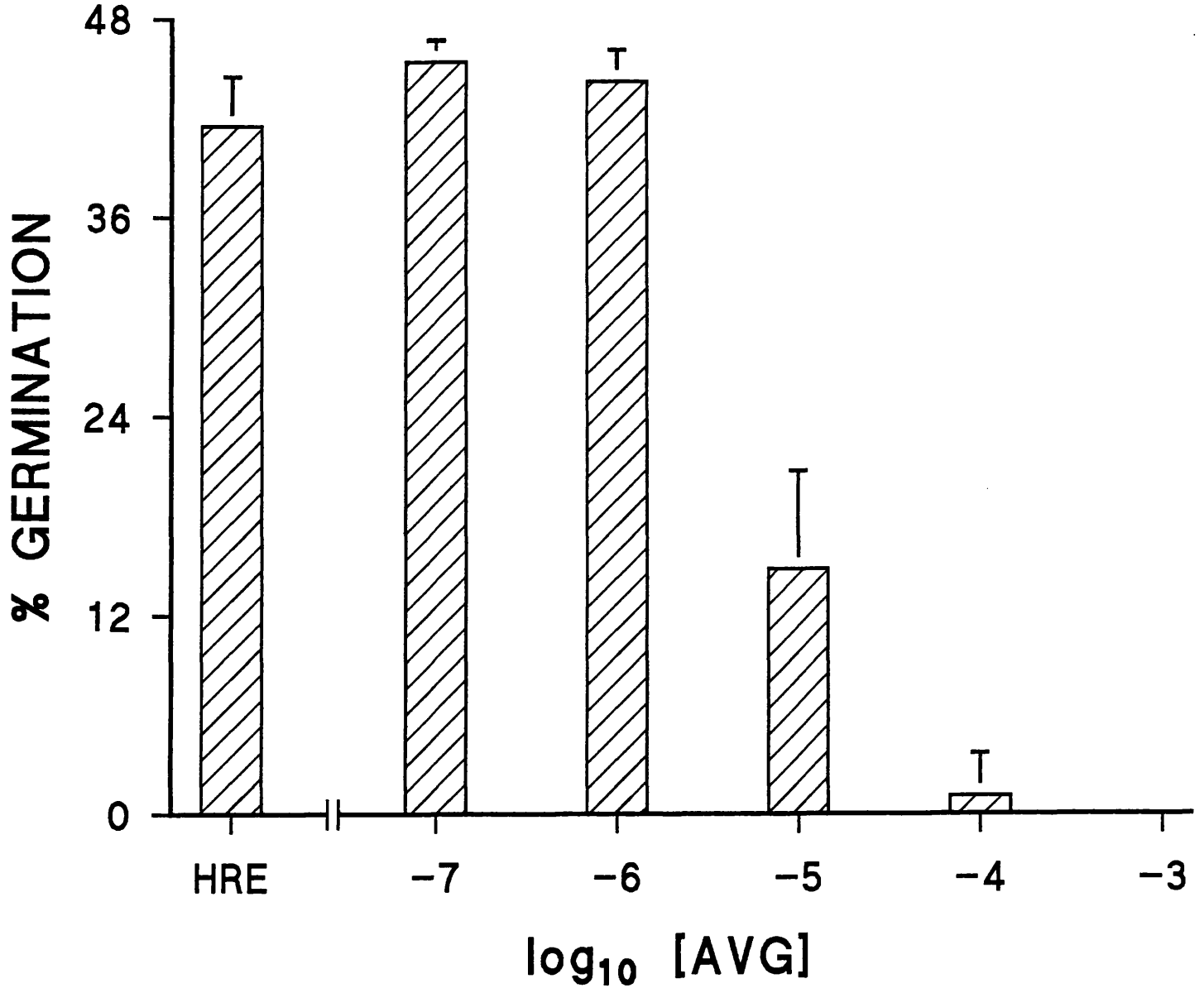
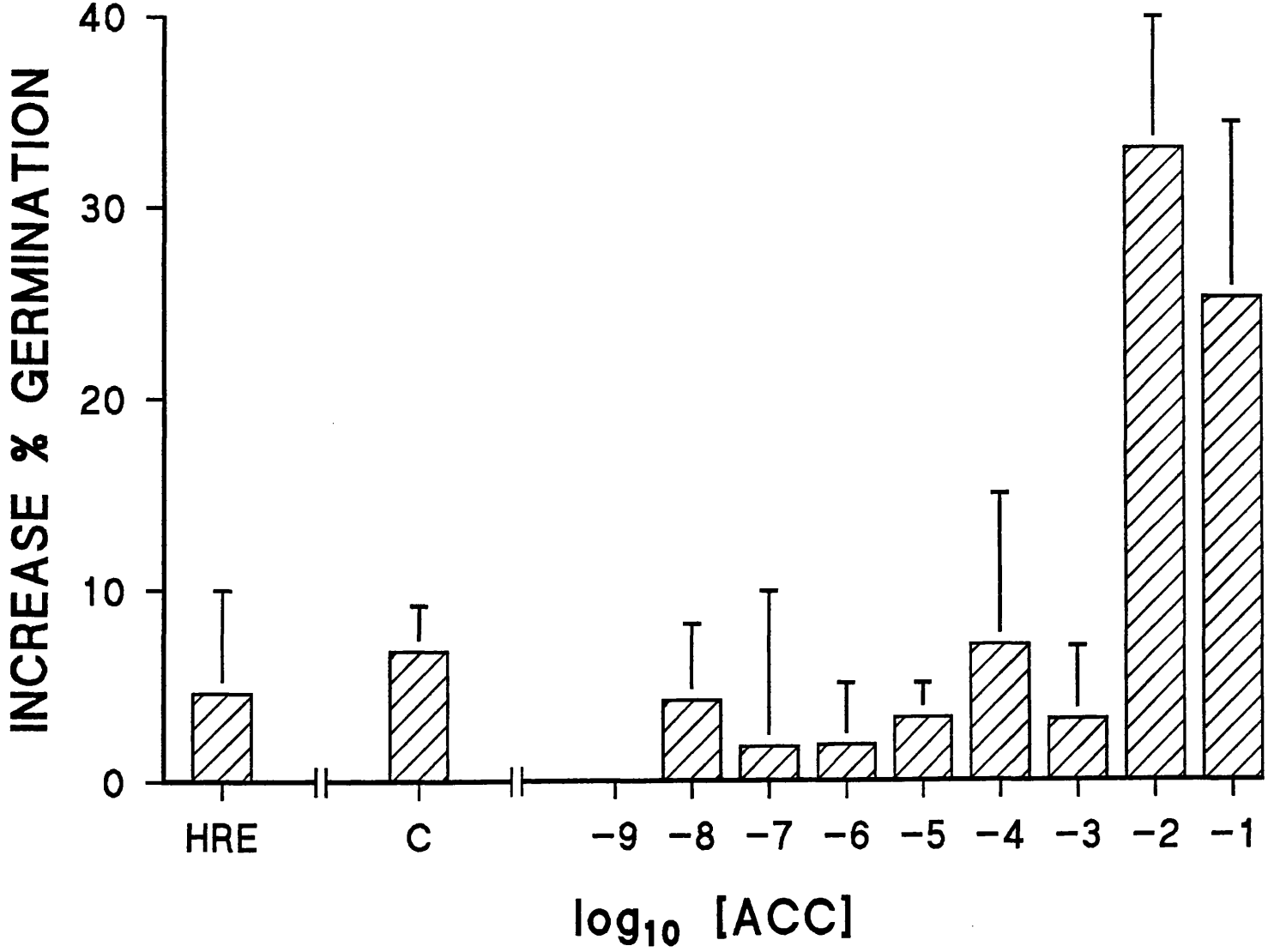

48
36
24
12
0
% GERMINATION
HRE
-7
-6
-5
-4
-3
log10 [AVG]

Figure 3.16

Overriding AVG inhibition of host root exudate stimulated germination by addition of ACC. *Striga hermonthica* seed collected in Wad Medani, Sudan in 1987.

Seeds were conditioned for 8 days before incubation for 24 h in host root exudate containing 10μM AVG. ACC was then added to the concentration shown. HRE - host root exudate only; C - host root exudate and 10μM AVG. Data are plotted as the percent increase in germination, over that measured at 24 h, a further 48 h later. Seeds incubated in distilled H_2O did not germinate. Error bars indicate S.D. (n=9).


INCREASE % GERMINATION
40
30
20
10
0
HRE
C
-9
-8
-7
-6
-5
-4
-3
-2
-1
log10 [ACC]

3.1.2.3 Stimulation by GR-24

The strigol analogue GR-24 stimulated germination of conditioned seeds (Fig. 3.17) although the response, as indicated by comparison of R_i, tG_{50} and G_{max} indices (Table 3.5), is lower than that obtained with host root exudate in all cases. However, the lag-time before radicle protrusion is similar at between 6 and 8 h. Seeds stimulated to germinate with GR-24 had a morphology identical to that of host root exudate stimulated seed except for the absence of root hairs (Figs. 3.5C and D; 3.6A and B).

3.1.2.4 Stimulation by ethylene

Incubating conditioned seeds with ethylene at 0.1μL/vial ($\approx$ 50μL/L) results in stimulation of germination with a pattern very similar to that caused by host root exudate (Fig 3.18A and Table 3.6). Increasing the concentration of ethylene 2.5 times did not change the percentage germination relative to that obtained with 0.1μL/vial (Fig. 3.18B). Seeds stimulated to germinated with ethylene at either concentration had a morphology indistinguishable from that of seeds stimulated with GR-24, no root hairs were present on the radicles (Fig. 3.5B).

3.1.2.5 Stimulation by thidiazuron

It has been known for many years that cytokinins could stimulate germination of conditioned *Striga* seeds (see 1.1.4.2). The cytokinin active urea derivative thidiazuron, stimulates germination in the absence of host root exudate (Fig. 3.19). The optimal concentration for germination is 1μM, increasing the

Figure 3.17

Effect of 3.4μM GR-24 on *Striga hermonthica* germination.

Seeds were conditioned for 7 days before transfer to 3.4μM GR-24. Each curve \represents a separate experiment. Seed incubated in s.H_2O instead of GR-24 did not germinate. Error bars indicate $\pm$ S.D. (n=12).

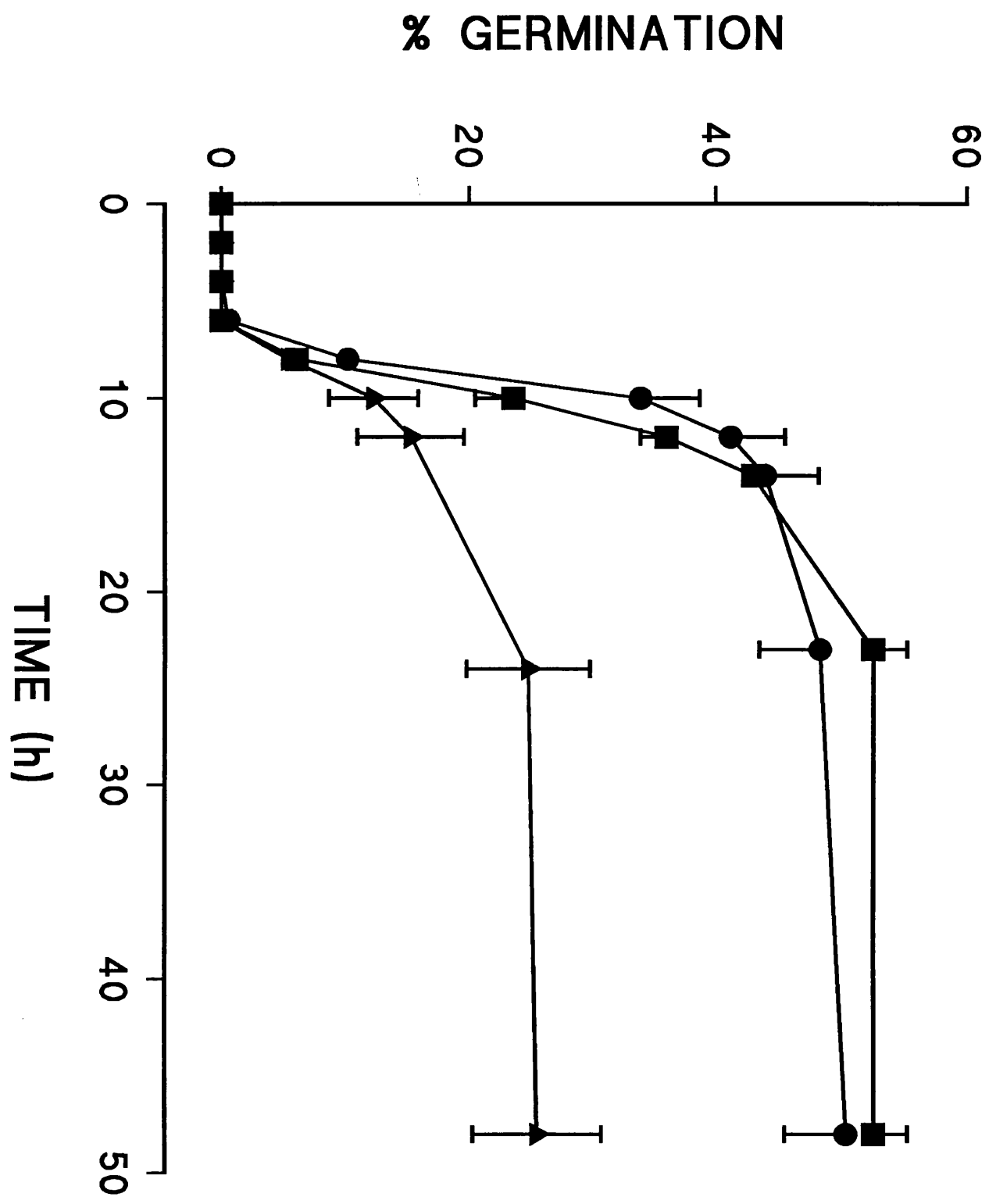

% GERMINATION
0
20
40
60
TIME (h)
0
10
20
30
40
50

Table 3.5

Germination indices for GR-24 stimulated germination. Data from Figure 3.17.

Exp.	R_i	tG_{50}	G_{max}
I	8.32	9.3	50.4
II	5.90	10.7	52.6
III	3.08	10.4	25.5
$\bar{X}$	5.77	10.1	42.8

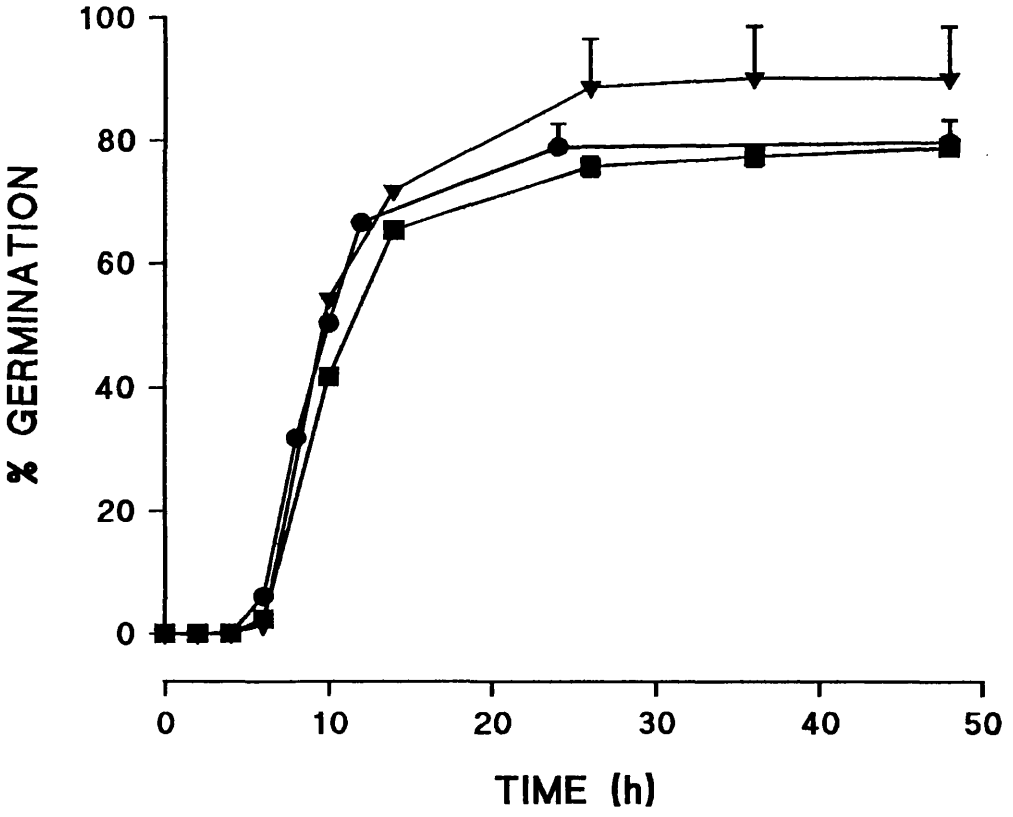
G_{max} - germination after 48h; R_i - initial germination rate; tG_{50} - time to 50% of G_{max}.

Figure 3.18 A, B

Effect of ethylene on the germination of 7 day conditioned *Striga hermonthica*.

Seeds were incubated with 0.1% (v/v) ethylene at two concentrations as described in 2.1.2, **A.** 100μL/vial; **B.** 250μL/vial. Each curve (**A** - ●, ■,▼; **B** - ●, ■) represents a separate experiment, Error bars indicate ± S.D. (n=12).

A


100
80
60
40
20
0
% GERMINATION
0
10
20
30
40
50
TIME (h)

B

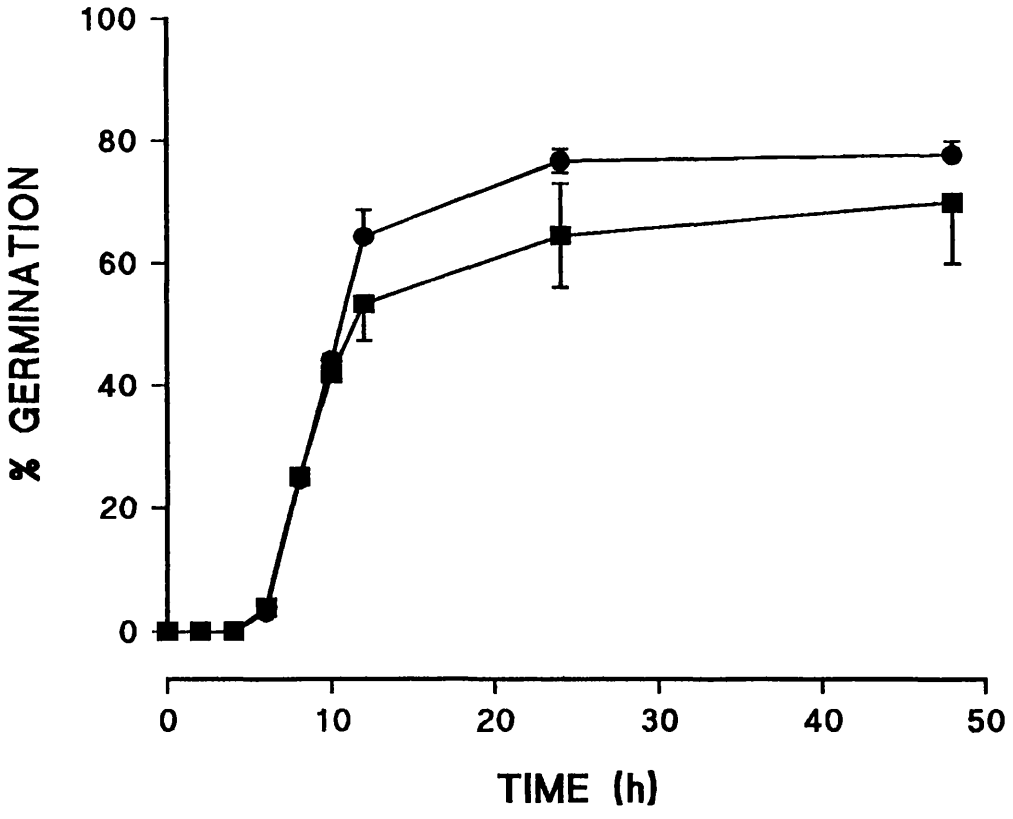

100
80
60
40
20
0
% GERMINATION
0
10
20
30
40
50
TIME (h)

Table 3.6

Germination indices for ethylene stimulated germination of conditioned seed. Data from Figure 3.18A.

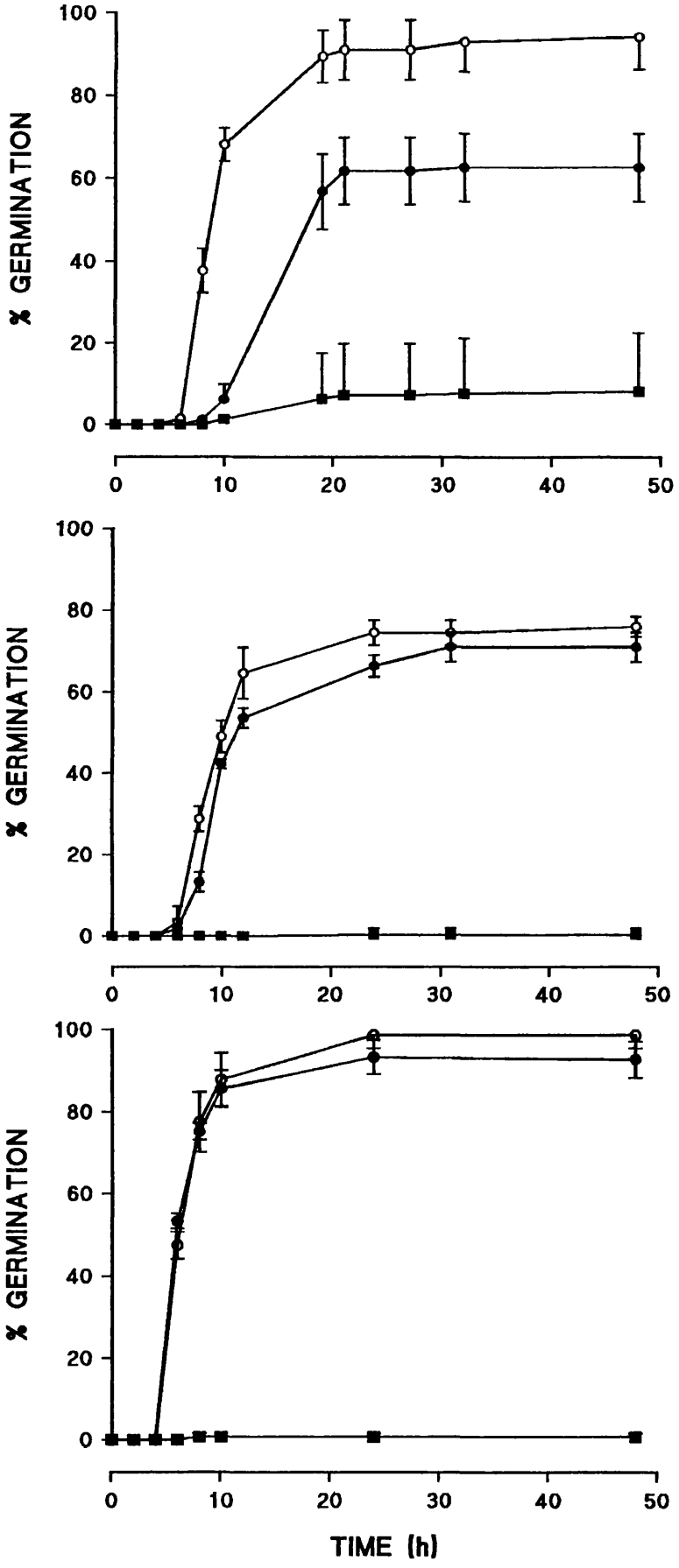
Exp.	R_i	tG_{50}	G_{max}
I	8.8	9.6	79.0
II	8.8	8.8	79.8
III	7.9	8.9	90.3
$\bar{X}$	8.51	9.1	83.0

G_{max} - germination after 48h; R_i - initial germination rate; tG_{50} - time to 50% of G_{max}.

Figure 3.19

Effect of thidiazuron on the germination of 7 day conditioned *Striga hermonthica* seed.

Seeds were incubated at zero time with ■, 10nM; ○, 1μM; or ●, 100μM thidiazuron. Error bars indicate ± S.D. (n=12). Each graph shows a separate experiment. Seed incubated in s.H_2O did not germinate.


% GERMINATION
100
80
60
40
20
0
0
10
20
30
40
50
% GERMINATION
100
80
60
40
20
0
0
10
20
30
40
50
% GERMINATION
100
80
60
40
20
0
0
10
20
30
40
50
TIME (h)

concentration to 100μM results in a reduction in germination percentage relative to that obtained with 1μM, with concentrations below 10nM being inactive. The response to 1μM is quite variable across the experimental replicates with R_i values from 11.4 to 19.37 (Table 3.7). Germination triggered by thidiazuron is partially inhibited by AVG, treatment with 1mM AVG, which completely inhibits germination stimulated by host root exudate, is no more effective in inhibiting thidiazuron stimulated germination than 50μM AVG (Fig. 3.20). As with stimulation by ACC, the response to thidiazuron can by inhibited by cobaltous ions (Table 3.8). Cobalt ions are a much more effective inhibitor of thidiazuron than ACC stimulated germination with 50mM, 10mM, and 1mM $CoCl_2$ all reducing germination to less than 5% in all but one experimental replicate (max = 12.2 $\pm$ 6.5). Thidiazuron stimulated germination is almost completely inhibited by NDE at 10μL stock/vial (Table 3.9).

Seeds stimulated with thidiazuron had a very distinct morphology. Radicles were much reduced in length with many root hair protuberances (Figs. 3.5G, 3.7). Over the first 24 h germination, the radicle swells but does not increase greatly in length. The seedling is usually free of the testa by 48 h germination, due to the development and growth of the cotyledons (Fig. 3.5G and 3.7B).

3.1.2.6 Stimulation by methionine, SAM or IAA

Treatment of conditioned seeds with methionine at concentrations

Table 3.7

Germination indices for thidiazuron (1μM) stimulated germination. Data from Fig. 3.19.

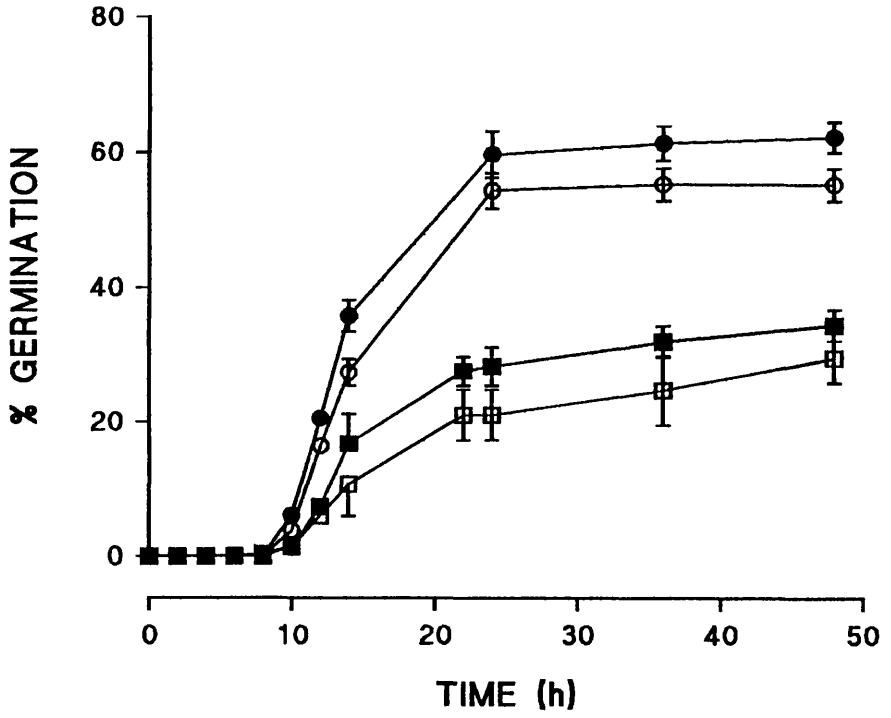
Exp.	R_i	tG_{50}	G_{max}
I	19.4	6.4	93.3
II	16.7	7.5	94.2
III	11.4	8.9	76.0
$\bar{X}$	15.8	7.6	87.8

G_{max} - germination after 48h; R_i - initial germination rate; tG_{50} - time to 50% of G_{max}.

Figure 3.20

Effect of AVG on thidiazuron stimulated germination of *Striga hermonthica.*

Seed were conditioned for 7 days before incubation in 1μM thidiazuron containing different concentrations of AVG: ●, 0; ○, 0.1μM; ■, 50μM; □, 1mM. Error bars indicate ± S.D. (n=12). Each graph represents a separate experiment.


80
60
40
20
0
% GERMINATION
0
10
20
30
40
50
TIME (h)

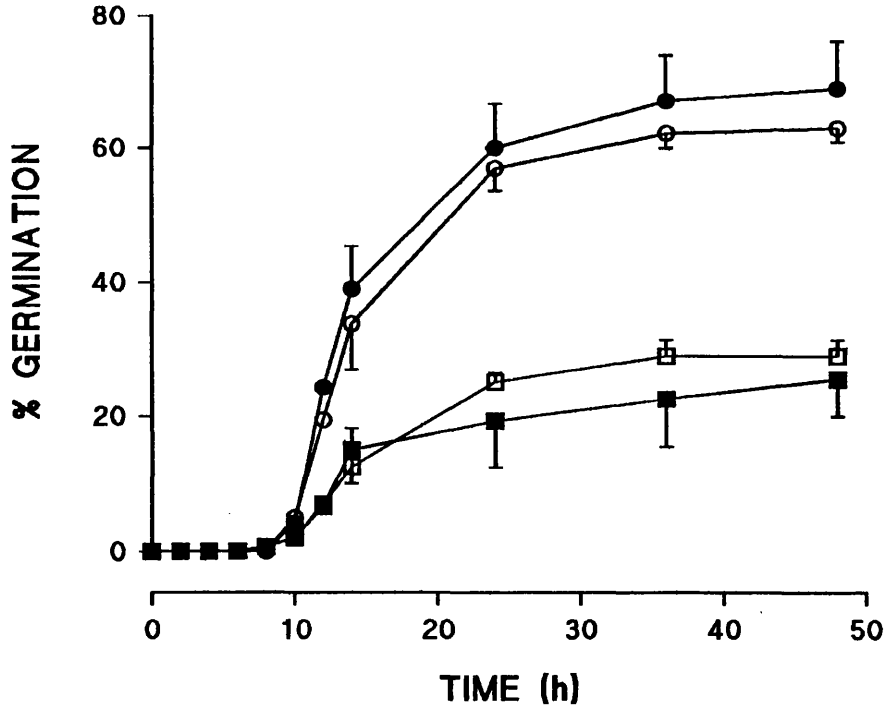

80
60
40
20
0
% GERMINATION
0
10
20
30
40
50
TIME (h)

Table 3.8

The effect of Co^{2+} on thidiazuron stimulated germination of 7 days conditioned seed.

Seeds of *Striga hermonthica* were conditioned for 7 days and incubated in thidiazuron (1μM) containing $CoCl_2$ (1, 10 or 50mM) as detailed in 2.1.1. Germination was measured after 48 h ($\pm$ S.D., n=12). Seed incubated in s.H_2O instead of thidiazuron did not germinate.

	% germination		
$[CoCl_2]$	Exp.		
	I	II	III
1mM	1.1 $\pm$ 2.5	12.2 $\pm$ 6.5	3.5 $\pm$ 5.9
10mM	4.8 $\pm$ 5.4	0.6 $\pm$ 2.1	3.9 $\pm$ 3.2
50mM	0.1 $\pm$ 0.9	0.1 $\pm$ 0.9	0.1 $\pm$ 0.9

Table 3.9

The effect of NDE on thidiazuron stimulated germination of 7 days conditioned seed.

Seeds of *Striga hermonthica* were conditioned for 7 days before incubation in 1μM thidiazuron and NDE as detailed in 2.1.1. Germination was measured after 48 h ($\pm$ S.D., n=12). Seed incubated in s.H_2O did not germinate.

% germination		
Exp.		
I	II	III
3.7 $\pm$ 4.6	0.8 $\pm$ 2.9	0

ranging from 2.5 to 250mM failed to induce germination greater than 4.2% (Table 3.10). Similarly, zero germination was obtained after treatment with IAA at concentrations from 0.1nM to 10mM. Incubation with SAM resulted in a germination maximum of 19.1 $\pm$ 4.4 % at 500μM in the first replicate and only 1.1 $\pm$ 2.5 % in the second replicate (Table 3.10).

3.1.3 Unconditioned seed (1989) - 24 h imbibition

Seeds given only 24 h imbibition are capable of germinating in response to ACC, thidiazuron and ethylene while the germination percentage following treatment with GR-24 or host root exudate is very low (Fig. 3.21 & Table 3.11). In comparison with germination triggered by these stimulants following 7 days conditioning, imbibition for 24 h results in germination being delayed, with a lag-time of at least 10 h. ACC shows the highest stimulation with G_{max} equal to that after 7 days conditioning. The response to both thidiazuron and ethylene is lower than that after 7 days conditioning. Thidiazuron treatment results in a G_{max} of 20-30% after 24 h conditioning compared to over 80% after 7 days.

ACC and thidiazuron stimulated germination of 24 h imbibed seed can be inhibited by Co^{2+} ions (Fig. 3.22 and Table 3.12). Inhibition of ACC stimulated germination increased with increasing concentration of Co^{2+}. 1mM $CoCl_2$ caused a reduction in G_{max} of over 50% while 50mM $CoCl_2$ was completely inhibitory. The germination of seed incubated with ACC at all $CoCl_2$ concentrations was atypical - the radicle protruding from the seed to a very limited extent (ca. 100μM) (Fig. 3.5F). Germination in response to thidiazuron was reduced to $<$0.5% by concentrations of $CoCl_2$ $\geq$1mM (Table 3.12).

Table 3.10

The effect of L-methionine, IAA or SAM on the germination of 7 day conditioned seed.

Seeds were conditioned for 7 days before incubation in either L-methionine, IAA or SAM as described in 2.1.1. Seed incubated in s.H_2O did not germinate. Germination was measured after 48 h (IAA and L-methionine) or 53 h (SAM) (± S.D., n=12).

Treatment	% Germination	
	Exp.	
	I	II
[L-methionine]		
2.5mM	0	0
25mM	0	0.1 ± 0.9
250mM	0	4.2 ± 5.1
[IAA]		
10mM	0	0
0.1mM	0.3 ± 1.5	0
1μM	0	0
10nM	0	0
0.1nM	0	0
[SAM]		
250μM	0	0
500μM	1.1 ± 2.5	19.1 ± 4.4
1mM	0	1.1 ± 2.5

Figure 3.21

Effect of GR-24, ACC, thidiazuron and ethylene on the germination of *Striga hermonthica* seed imbibed for 24 h.

Seeds were imbibed in s.H_2O for 24 h prior to incubation at time zero in: ●, 50mM ACC; ■, 1μL/vial ethylene; ○, 1μM thidiazuron; □, 3.4μM GR-24. Error bars indicate ± S.D. (n=12). Each graph shows a separate experiment. Seed incubated in s.H_2O did not germinate.

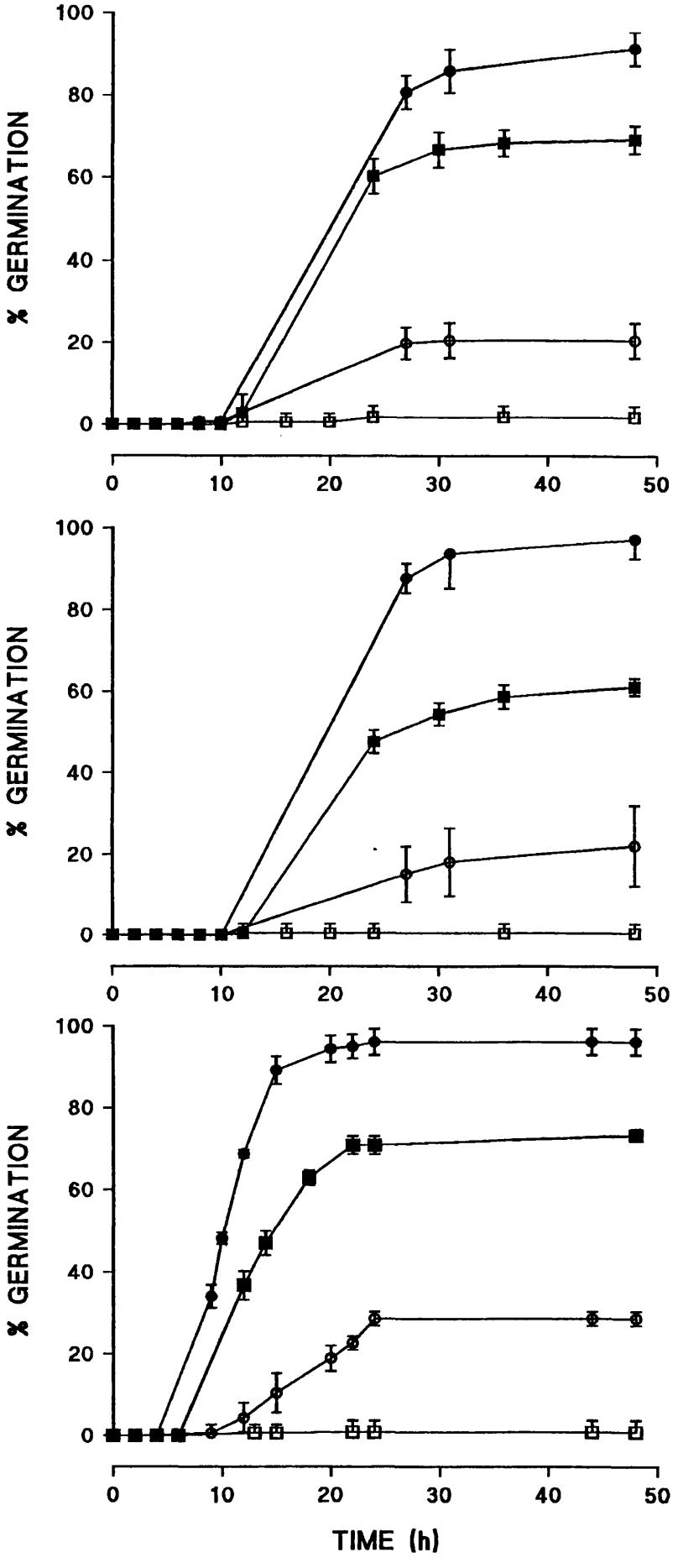

% GERMINATION
100
80
60
40
20
0
0
10
20
30
40
50
% GERMINATION
100
80
60
40
20
0
0
10
20
30
40
50
% GERMINATION
100
80
60
40
20
0
0
10
20
30
40
50
TIME (h)

Table 3.11

Comparison between host root exudate and ACC stimulation of germination and ethylene production by 24 h or 7 day conditioned seed.

Seeds conditioned for either 24 h or 7 days were incubated in host root exudate (HRE) or 10mM ACC. The same host root exudate preparation was used for all treatments. Germination was determined at 48 h (± S.D., n=12) while ethylene concentrations were measured at 6 h (± S.E., n=3).

	% Germination		Ethylene nL $vial^{-1}$ $gram^{-1}$	
	HRE	ACC	HRE	ACC
24 h	10.0 ± 4.3	81.0 ± 1.9	N/D	170 ± 27
7 day	95.0 ± 6.5	93.1 ± 3.3	-	1082 ± 32

Figure 3.22

Inhibition by $CoCl_2$ of the germination of 24 h imbibed *Striga hermonthica* seeds incubated with ACC.

Seeds were imbibed for 24 h before incubation in 50mM ACC containing different concentrations of $CoCl_2$: ●, 1mM; ○, 10mM; ■, 50mM. Error bars indicate ± S.D. (n=12). Each graph shows a separate experiment. Seed incubated in s.H_2O instead of ACC did not germinate.

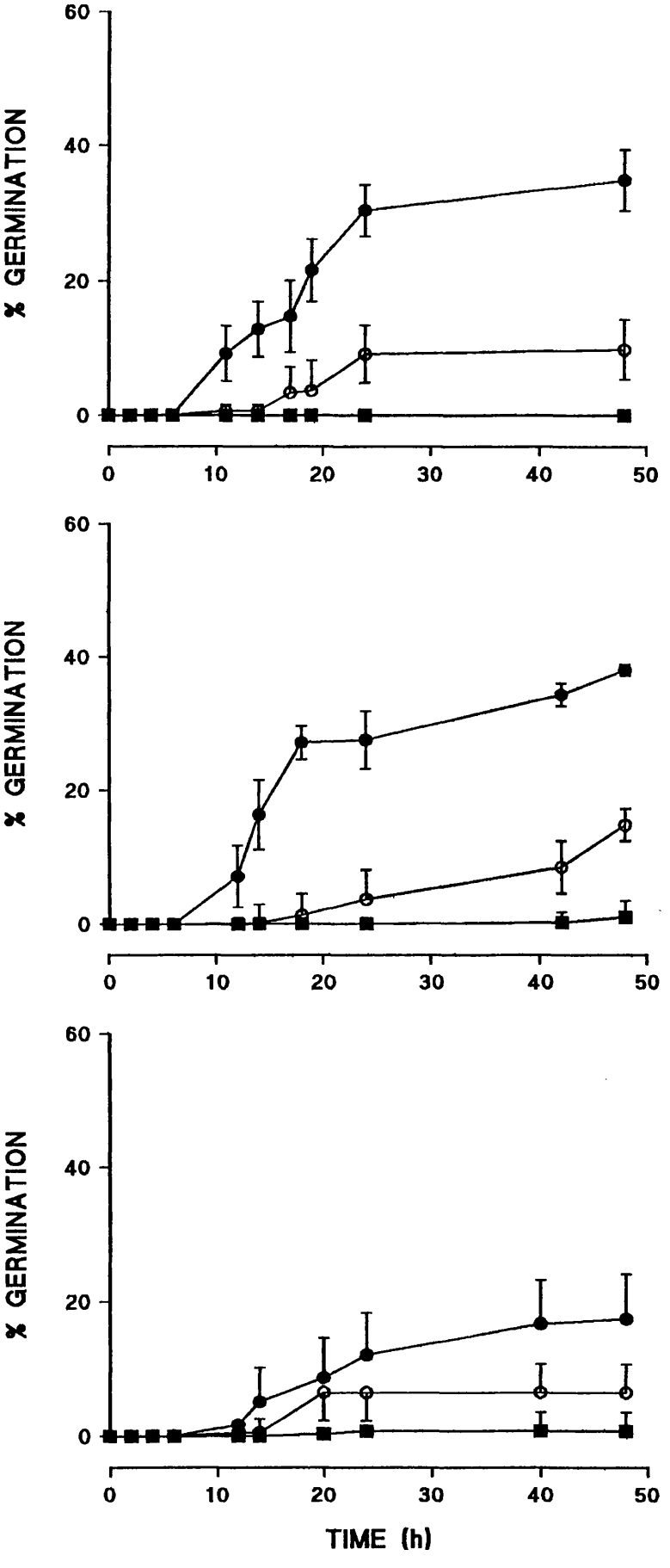

% GERMINATION
60
40
20
0
0
10
20
30
40
50
% GERMINATION
60
40
20
0
0
10
20
30
40
50
% GERMINATION
60
40
20
0
0
10
20
30
40
50
TIME (h)

Table 3.12

The effect of Co^{2+} on thidiazuron stimulated germination of 24 h imbibed seed.

Seeds of *Striga hermonthica* were conditioned for 24 h and incubated in thidiazuron (1μM) containing $CoCl_2$ (1, 10 or 50mM) as detailed in 2.1.1. Germination was measured after 48 h ($\pm$ S.D., n=12). Seed incubared in s.H_2O instead of thidiazuron did not germinate.

	% germination		
[$CoCl_2$]	Exp.		
	I	II	III
1mM	0.1 $\pm$ 0.9	0.1 0.9	0.1 $\pm$ 0.9
10mM	0	0.1 $\pm$ 0.9	0
50mM	0	0.3 $\pm$ 1.6	0

3.2 Ethylene production

3.2.1 Conditioned seed - 7 days imbibition

3.2.1.1 Host root exudate

Ethylene is produced rapidly upon treatment of *S. hermonthica* seeds with host root exudate, reaching a maximum after 9 h (Fig. 3.23). Similar results were obtained with *S. hermonthica* seed collected from the same area in 1981 (Fig. 3.23) although ethylene production peaked earlier, at 6 h. Incubation in s.H_2O produces no detectable ethylene after 24 h. Plotting % germination alongside ethylene production shows clearly that ethylene production precedes visible germination (Fig. 3.24).

Incubation with AVG and host root exudate causes a marked reduction in the amount of ethylene produced while application of NDE in the presence of host root exudate causes a slight stimulation of ethylene production (Table 3.13). Seeds collected from Wad Medani, Sudan in 1981 showed a similar response, with 10μM AVG inhibiting host root exudate stimulated ethylene production by over 60% (Table 3.13).

Conditioning seeds for only 24 h results in no detectable ethylene production following stimulation with host root exudate (Table 3.11).

3.2.1.2 GR-24

Stimulation of ethylene production by GR-24 is much more rapid than that as a result of host root exudate treatment (Fig. 3.25). Measurable ethylene is produced by 3 h in response to GR-24 while that produced by seeds stimulated with host root exudate is zero. After 6 h, coincident with radicle protrusion, the

Figure 3.23

Time course of ethylene production by *Striga hermonthica* seeds collected from Wad Medani, Sudan in 1989 (●) or 1981 (■) during incubation with host root exudate.

Seeds were conditioned for 2 (1981) or 7 (1989) days before transfer to host root exudate at time zero. Error bars indicate ± S.E. (n=3). Seeds incubated in double distilled deionised water produced no detectable ethylene after 24 h incubation.

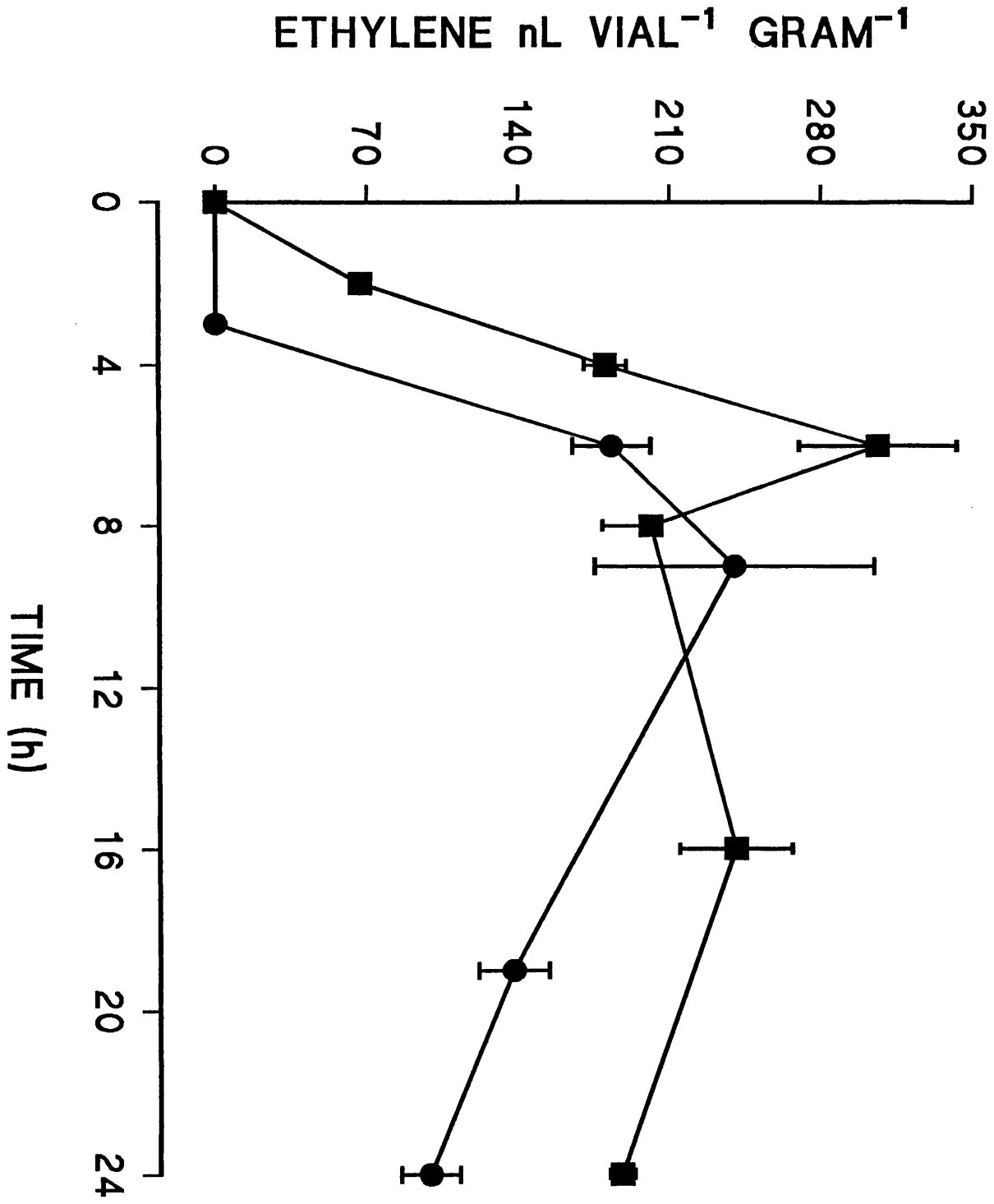

ETHYLENE nL VIAL^{-1} GRAM^{-1}
0
70
140
210
280
350
TIME (h)
0
4
8
12
16
20
24

Figure 3.24

Time course of *Striga hermonthica* seed germination (●, ± S.D., n=12) and ethylene production (□, ± S.E., n=3) during incubation with host root exudate.

Seeds were conditioned for 7 days before transfer to host root exudate at time zero.

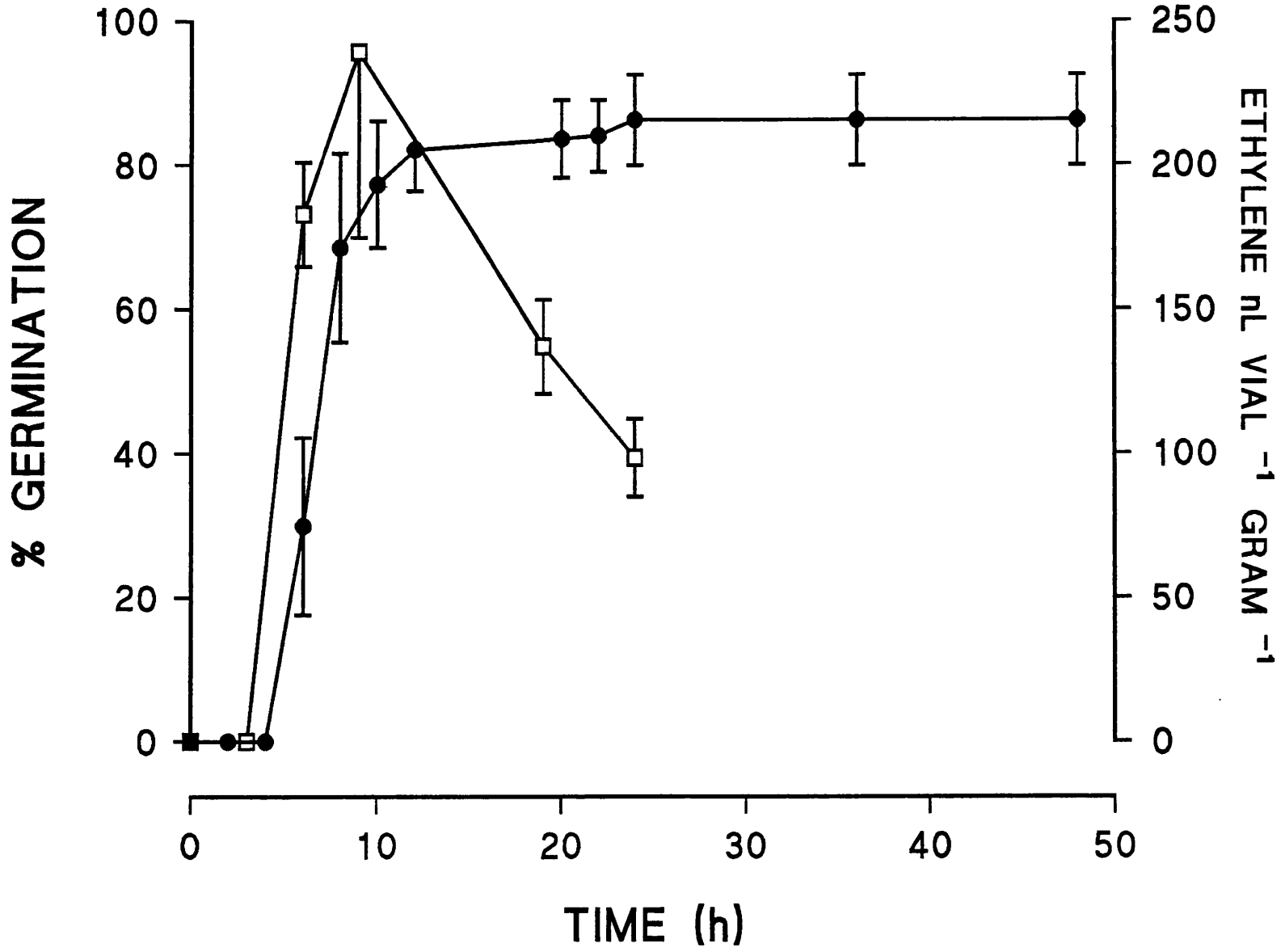

% GERMINATION
ETHYLENE nL VIAL -1 GRAM -1
TIME (h)
100
80
60
40
20
0
250
200
150
100
50
0
0
10
20
30
40
50

Table 3.13

Effect of ACC, AVG or NDE on ethylene production.

Seed was conditioned for 2 (1981) or 7 (1989) days before transfer to vials at time zero. For seed from Wad Medani, 1989 measurements were first made at 6 h, the vials were then re-equilibrated with air at 33°C, returned to the incubator and sampled 18 h (50μM AVG) or 24 h later. Values are means ± S.E., n=3; those in brackets are complete experimental replicates. HRE= host root exudate.

Ethylene nL $vial^{-1}$ $gram^{-1}$

Wad Medani 1981

Treatment	6 hours	12 hours	24 hours
HRE	284 ± 11 (306 ± 36)	138 ± 22	187 ± 6
" + 10μM AVG	103 ± 4	42 ± 13	
10mM ACC	833 ± 123	-	
Distilled H_2O			N/D

Wad Medani 1989

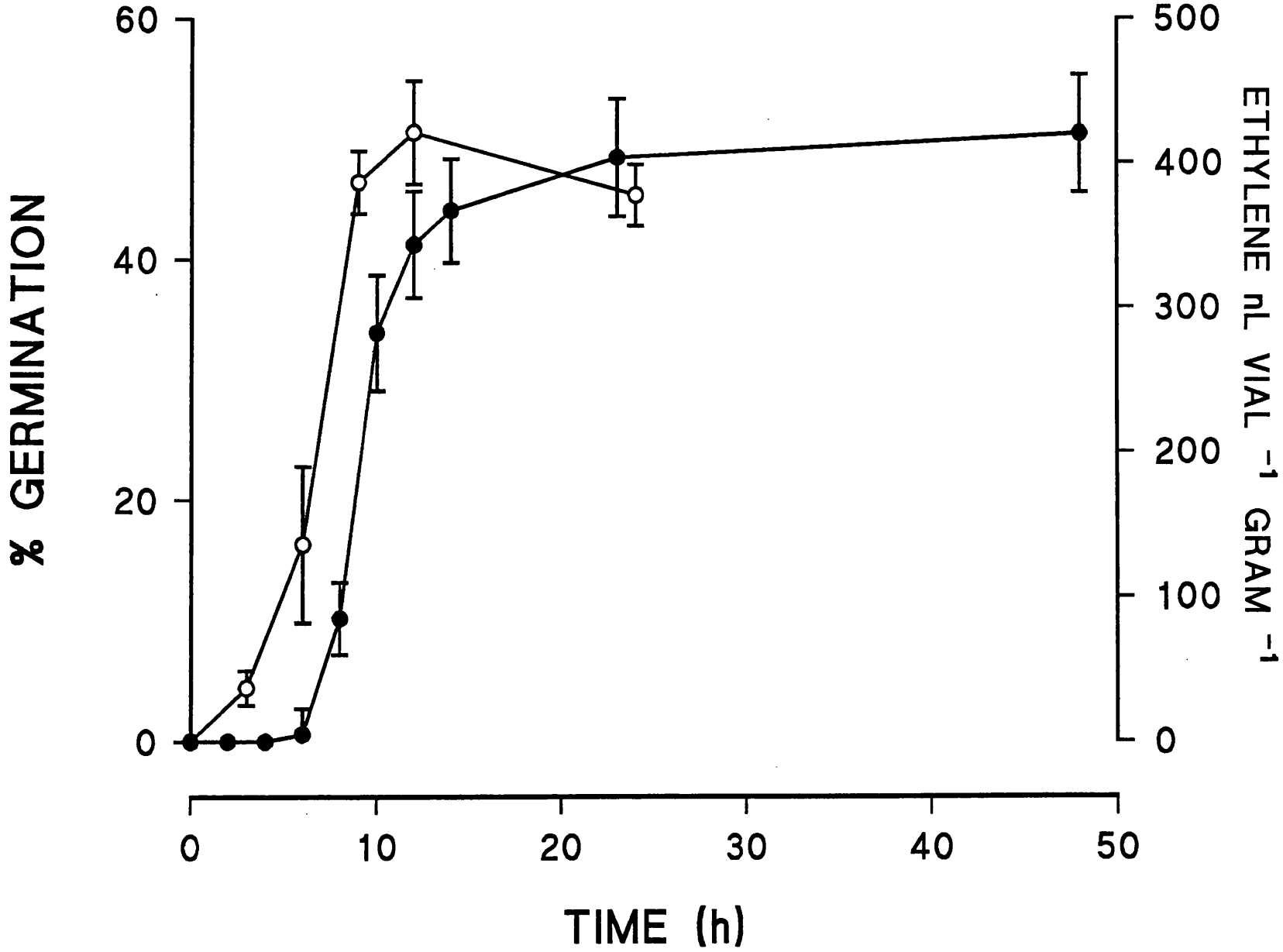
Treatment	6 hours	24 hours	30 hours
HRE	186 ± 19 (189 ± 5)	101 ± 14	111 ± 8
" + 1mM AVG	N/D	-	N/D
" + 50μM AVG	N/D	15 ± 5	-
" + NDE	274 ± 10	-	163 ± 7
10mM ACC	1082 ± 32	-	6256 ± 9
100μM ACC	N/D	-	68 ± 10
s.H_2O	N/D	-	N/D

N/D = not detected

Figure 3.25

Time course of *Striga hermonthica* seed germination (●, ± S.D., n=12) and ethylene production (□, ± S.E., n=3) during incubation with 3.4μM GR-24.

Seeds were conditioned for 7 days before transfer to 3.4μM GR-24 at time zero.


% GERMINATION
60
40
20
0
500
400
300
200
100
0
ETHYLENE nL VIAL -1 GRAM -1
0
10
20
30
40
50
TIME (h)

amounts of ethylene produced are comparable (136 ± 54 nL $vial^{-1}$ $gram^{-1}$ for GR-24 compared to 187 ± 34 nL $vial^{-1}$ $gram^{-1}$ (± S.E.) for host root exudate) (Fig. 3.23). Incubation with GR-24 caused a greater stimulation of ethylene production than incubation with host root exudate, 386.4 ± 37.8 compared to 239.4 ± 64.6 nL $vial^{-1}$ $gram^{-1}$ (± S.E.) after 9 h incubation. However, unlike host root exudate, treatment with GR-24 does not lead to a rapid decline in the amount of ethylene within the vial although the amount does decrease from 421 ± 20.8 to 377.2 ± 12.3 nL $vial^{-1}$ $gram^{-1}$ (± S.E.).

Incubating in the presence of AVG results in a reduction in the amount of ethylene produced in a concentration dependant manner with 1mM AVG completely inhibiting ethylene production (Table 3.14). Returning the vials to the incubator after equilibration with laboratory air at 33°C, and sampling 24 h later reveals that there is still zero detectable ethylene in the vial containing GR-24 and 1mM AVG whereas with GR-24 and no AVG the ethylene concentration in the vial has returned to a level similar to that at 9 h (Table 3.14).

Incubation in the presence of the ethylene action inhibitor NDE, slightly stimulates ethylene production as found for host root exudate and NDE treatment (Table 3.14).

3.2.1.3 ACC

Incubation with 10mM ACC massively stimulates ethylene production while stimulation by 100μM is only apparent after 24 h (Table 3.13). Ethylene production is stimulated by treatment with 10mM ACC after only 24 h imbibition although the amount produced is much smaller than that after the full 7 days conditioning (Table 3.11).

Table 3.14

The effect of AVG or NDE on GR-24 stimulated ethylene production.

Seeds were conditioned for 7 days before incubation in 3.4μM GR-24 containing AVG or NDE as detailed in 2.1.2. Ethylene concentrations were determined after 6 or 9 h, the vials were then re-equilibrated and sampled after a further 18 or 15 h respectively ($\pm$ S.E., n=3).

Treatment	Ethylene nL vial^{-1} gram^{-1}		
	6 hours	9 hours	24 hours
GR-24	136 $\pm$ 54	246 $\pm$ 72	-
" + 50μM	-	24 $\pm$ 8	34 $\pm$ 25
" + 1mM	-	N/D	-
" + NDE	185 $\pm$ 61	-	331 $\pm$ 55

3.2.1.4 Thidiazuron

Incubation of seeds with thidiazuron results in stimulation of ethylene production (Fig. 3.26). However, in contrast to stimulation by host root exudate and GR-24, initiation of measureable ethylene production follows radicle protrusion (Fig. 3.26). Although germination had already reached over 28% in the slowest responding experimental replicate after 8 h measurable ethylene was still zero at 9 h (Fig. 3.19).

Stimulation of ethylene production by thidiazuron can be inhibited by both AVG and NDE (Table 3.15). AVG at 50μM is completely inhibitory while NDE causes a massive reduction in ethylene production.

3.3 Respiration

3.3.1 Conditioning

As shown in Fig 3.27, seed of *Striga hermonthica* collected from Wad Medani in 1981 respire at 8.9 $\pm$ 1.8 μmoles h^{-1} g^{-1} ($\pm$ S.E.) over the first hours imbibition at 33°C. As the conditioning time increases, O_2 uptake shows a statistically significant peak of 32.8 $\pm$ 9.6 μmoles h^{-1} g^{-1} ($\pm$ S.E.) at 2 days, the rate then fluctuates, between an average of 10 to 25μmoles h^{-1} g^{-1}, over the next 12 days. Seed collected from the same area in 1989 shows a similar pattern of O_2 uptake to that from 1981; with an average rate over the first hours imbibition of 6.8 $\pm$ 1.9, rising to 18.6 $\pm$ 8.6 μmoles h^{-1} g^{-1} ($\pm$ S.E.) 24 h later (Fig. 3.28). As with seed collected in 1981, the rate remains constant at around 20μmoles h^{-1} g^{-1} over the following 10 days.

Figure 3.26

Time course of *Striga hermonthica* seed germination (●, ± S.D., n=12) and ethylene production (□, ± S.E., n=3) during incubation with 1μM thidiazuron.

Seeds were conditioned for 7 days before transfer to 1μM thidiazuron at time zero.

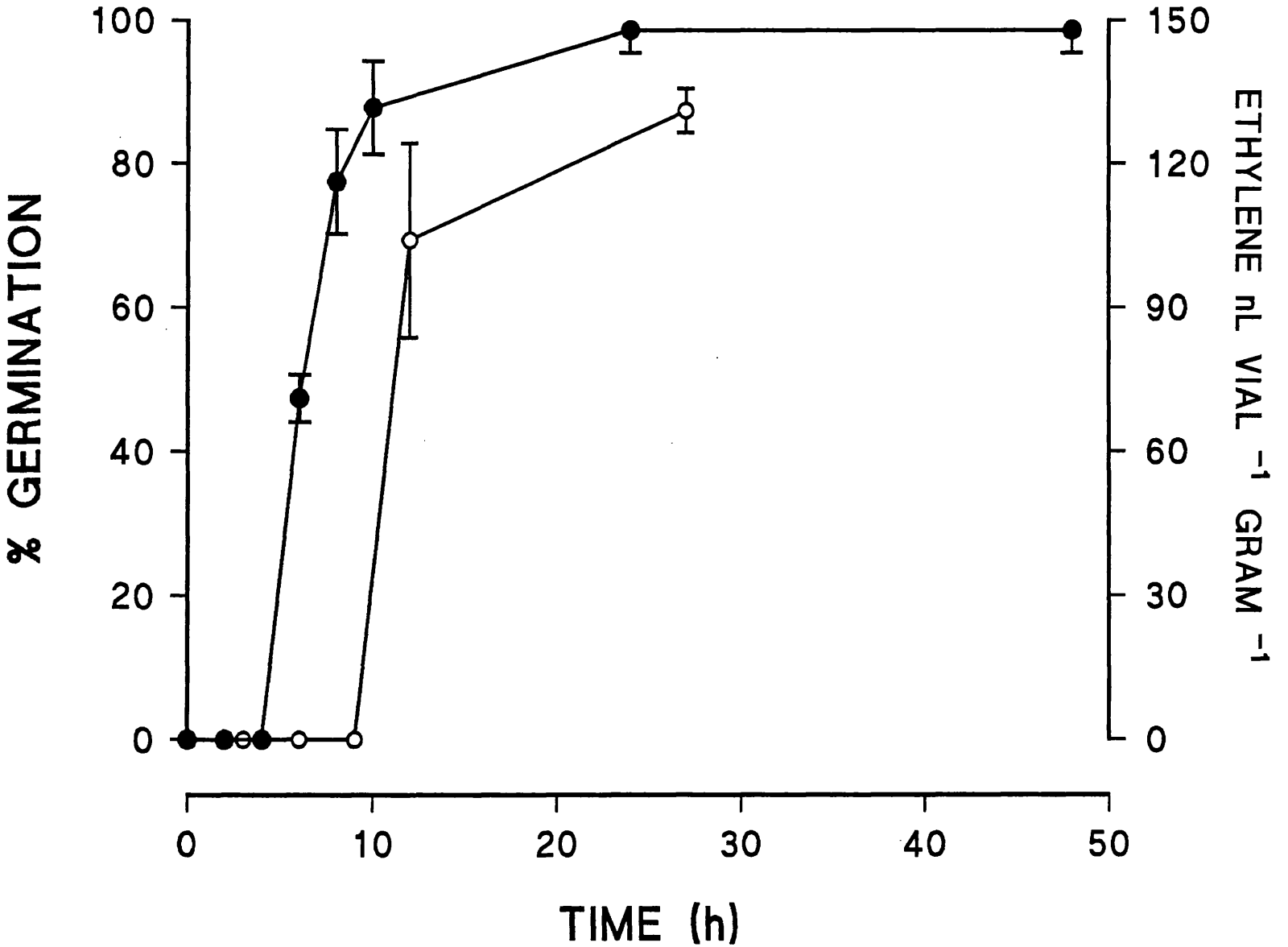

% GERMINATION
100
80
60
40
20
0
150
120
90
60
30
0
ETHYLENE nL VIAL -1 GRAM -1
0
10
20
30
40
50
TIME (h)

Table 3.15

The effect of AVG or NDE on thidiazuron stimulated ethylene production.

Seeds were conditioned for 7 days before incubation in 1μM thidiazuron containing AVG or NDE as detailed in 2.4 ($\pm$ S.E., n=3).

Treatment	Ethylene nL $vial^{-1}$ $gram^{-1}$	
	24 h	27 h
thidiazuron	-	131 $\pm$ 5
" + 50μM AVG	N/D	-
" + NDE	14 $\pm$ 5	-

Figure 3.27

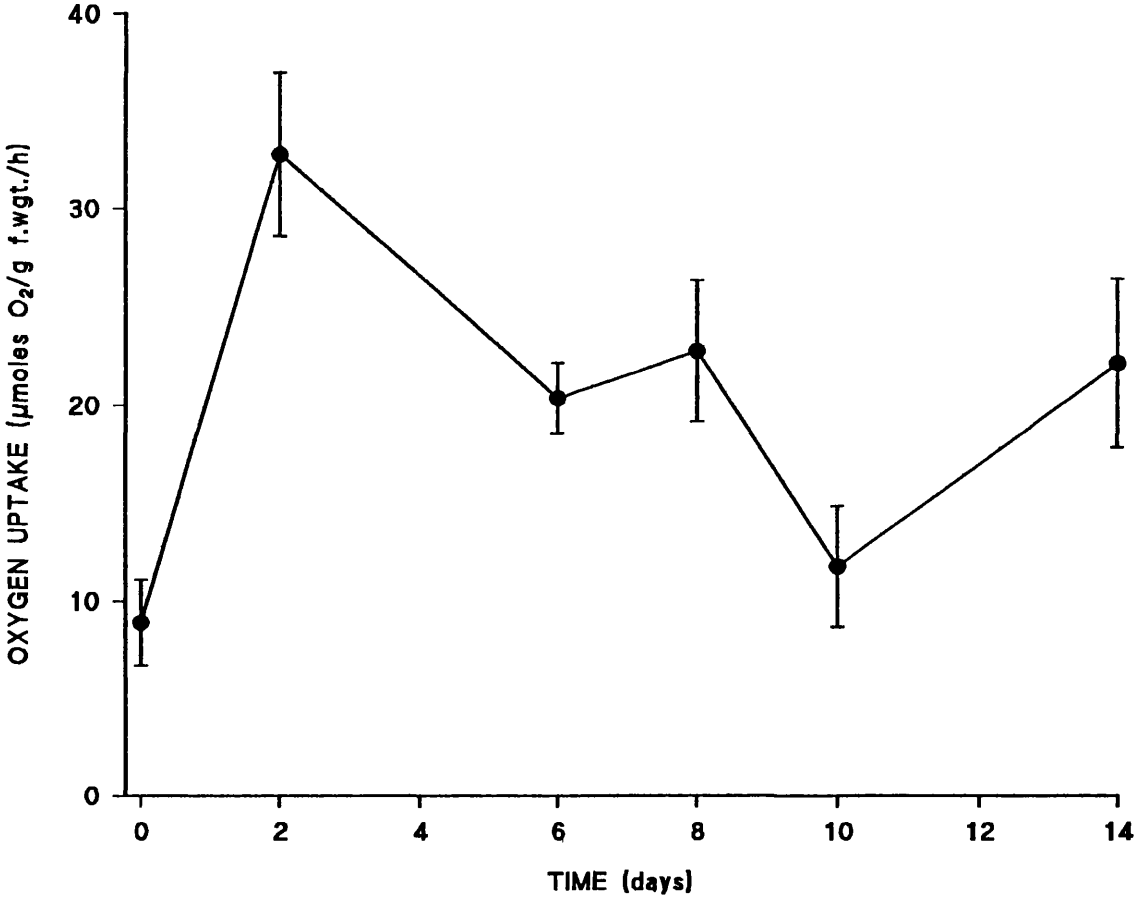
Effect of length of conditioning on O_2 uptake by *Striga hermonthica* seeds collected from Wad Medani, Sudan in 1981.

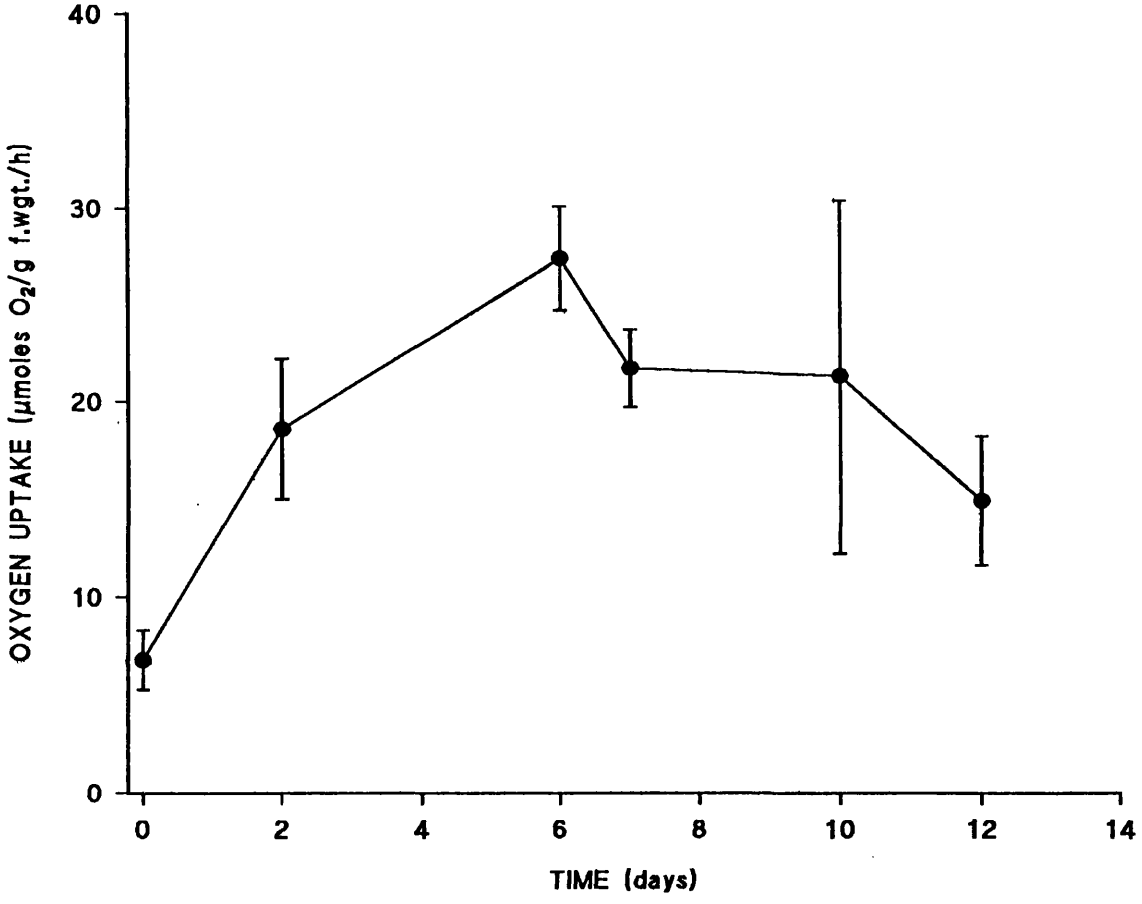
Seeds were conditioned for the time indicated before measurement of O_2 uptake for 1 h. Error bars indicate $\pm$ 95% confidence intervals.

Figure 3.28

Effect of length of conditioning on O_2 uptake by *Striga hermonthica* seeds collected from Wad Medani, Sudan in 1989.

Seeds were conditioned for the time indicated before measurement of O_2 uptake for 1 h. Error bars indicate $\pm$ 95% confidence intervals.


OXYGEN UPTAKE (µmoles O_2/g f.wgt./h)
40
30
20
10
0
0
2
4
6
8
10
12
14
TIME (days)


OXYGEN UPTAKE (µmoles O_2/g f.wgt./h)
40
30
20
10
0
0
2
4
6
8
10
12
14
TIME (days)

3.3.2 Stimulation by GR-24, ACC, ethylene and thidiazuron

The time course of O_2 uptake by 7 day conditioned *S. hermonthica* seed, challenged with GR-24, shows a lag period of 12 h before there is a significant increase in the rate of O_2 uptake above the basal rate attributable to the conditioning period (Fig. 3.29). The rate increases rapidly over the next 12 h to 261.5 ± 33.1 μmoles h^{-1} g^{-1} after 24 h incubation in 3.4μM GR-24. When the conditioning time is reduced to 24 h there is no significant increase in respiratory activity following the addition of GR-24; the rate of O_2 uptake remains constant at between 20 to 30μmoles h^{-1} g^{-1}.

In contrast to treatment with GR-24, treatment with ACC after 7 days conditioning results in a rapid increase in O_2 uptake from a basal level of 18.7 ± 1.0 at time zero to 25.8 ± 1.2 μmoles h^{-1} g^{-1} over the first 15 min after the addition of ACC to 50mM (Fig. 3.31). The rate increases steadily over the subsequent 6 h, by which time radicle protrusion was detectable, before a burst of respiration during the next 6 h. The increase in O_2 uptake from 115.7 ± 8.1 at 12 h to 125.1 ± 4.6 μmoles h^{-1} g^{-1} at 24 h is not statistically significant. This rate is, however, less than half the rate obtained following incubation with GR-24.

Conditioning seed for 24 h before incubation with 50mM ACC results in a different pattern of O_2 uptake to that observed with seed conditioned for 7 days. Addition of ACC does not cause a significant increase in respiration within the first hour and the rate of O_2 uptake after 12 h stimulation is approximately 50% less. However, after 24 h incubation the rates observed with seed conditioned for either 24 h or 7 days were not significantly different.

Treatment with ethylene induces a similar rapid increase in O_2 uptake in 7

Figure 3.29

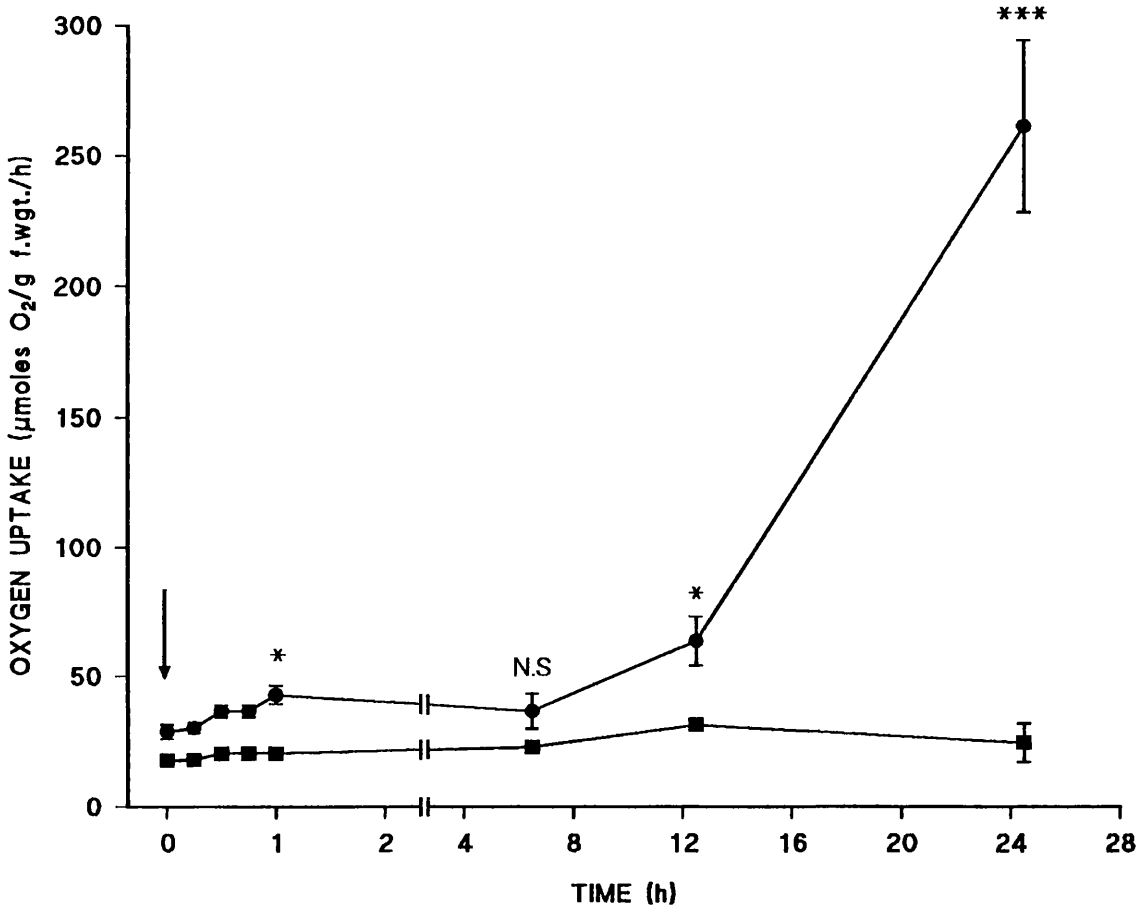
Effect of 3.4μM GR-24 on the O_2 uptake of *Striga hermonthica* seeds.

Seeds were either ■, imbibed for 24 h; or ●, conditioned for 7 days before measurement of O_2 uptake for 1 h at the times indicated. Arrow indicates the addition of GR-24 to 3.4μM. Error bars indicate ± S.E.; stars indicate a significant difference from the rate prior to addition of GR-24; * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $P \leq 0.001$, N.S. = not significant.

Figure 3.30

Effect of 1μM thidiazuron on the O_2 uptake of *Striga hermonthica* seeds.

Seeds were conditioned for 7 days before measurement of O_2 uptake for 1 h at the times indicated. Arrow indicates the addition of thidiazuron to 1μM. Error bars indicate ± S.E.; stars indicate a significant difference from the rate prior to addition of thidiazuron; * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $P \leq 0.001$, N.S. = not significant.


300
250
200
150
100
50
0
OXYGEN UPTAKE (µmoles O_2/g f.wgt./h)
*
N.S
*
***
0
1
2
4
8
12
16
20
24
28
TIME (h)

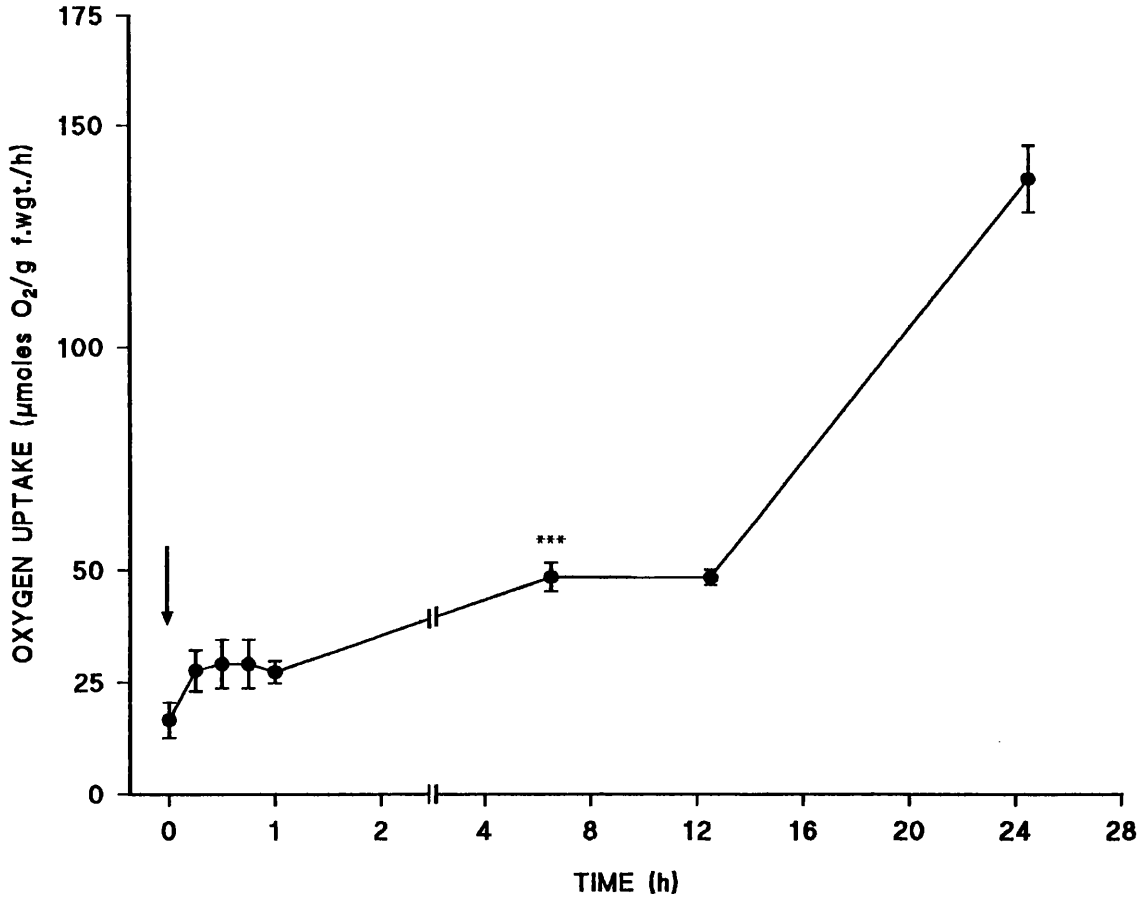

175
150
100
50
25
0
OXYGEN UPTAKE (µmoles O_2/g f.wgt./h)
***
0
1
2
4
8
12
16
20
24
28
TIME (h)

Figure 3.31

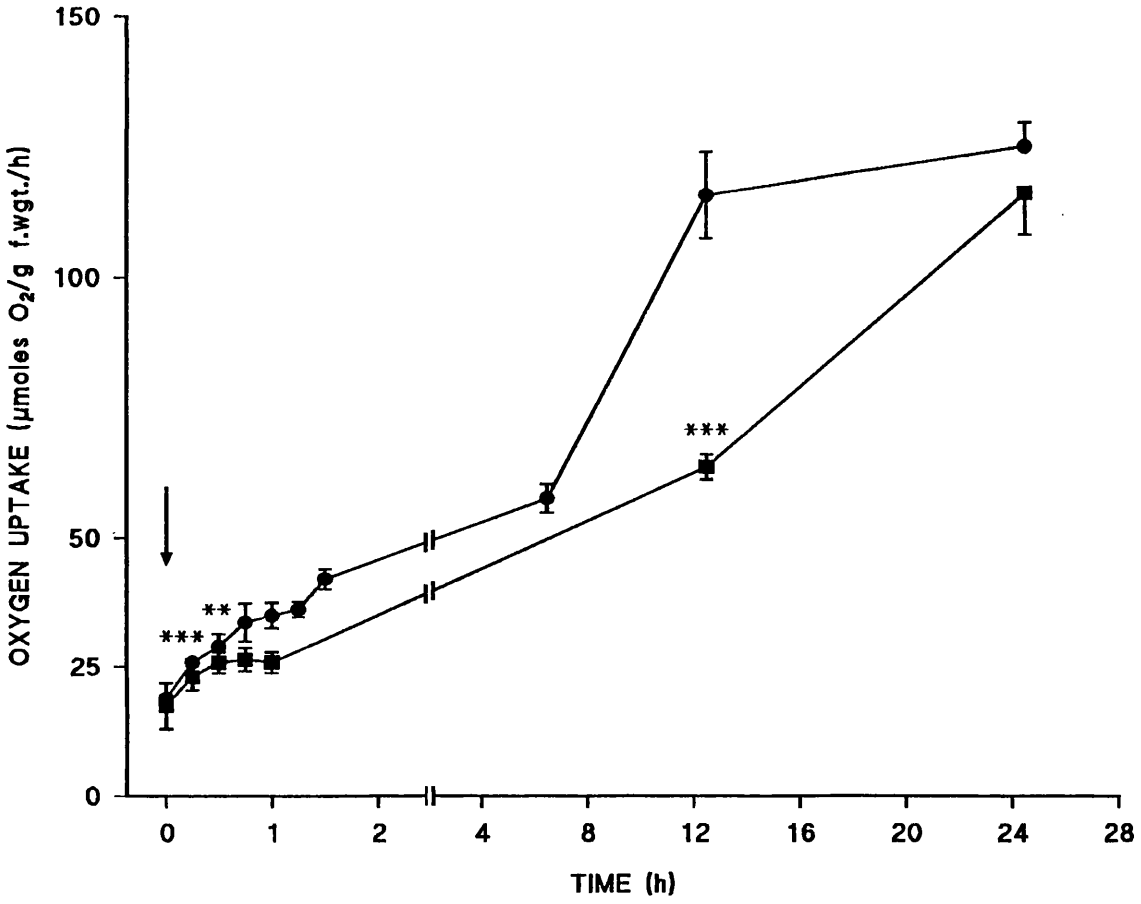
Effect of 50mM ACC on the O_2 uptake of *Striga hermonthica* seeds.

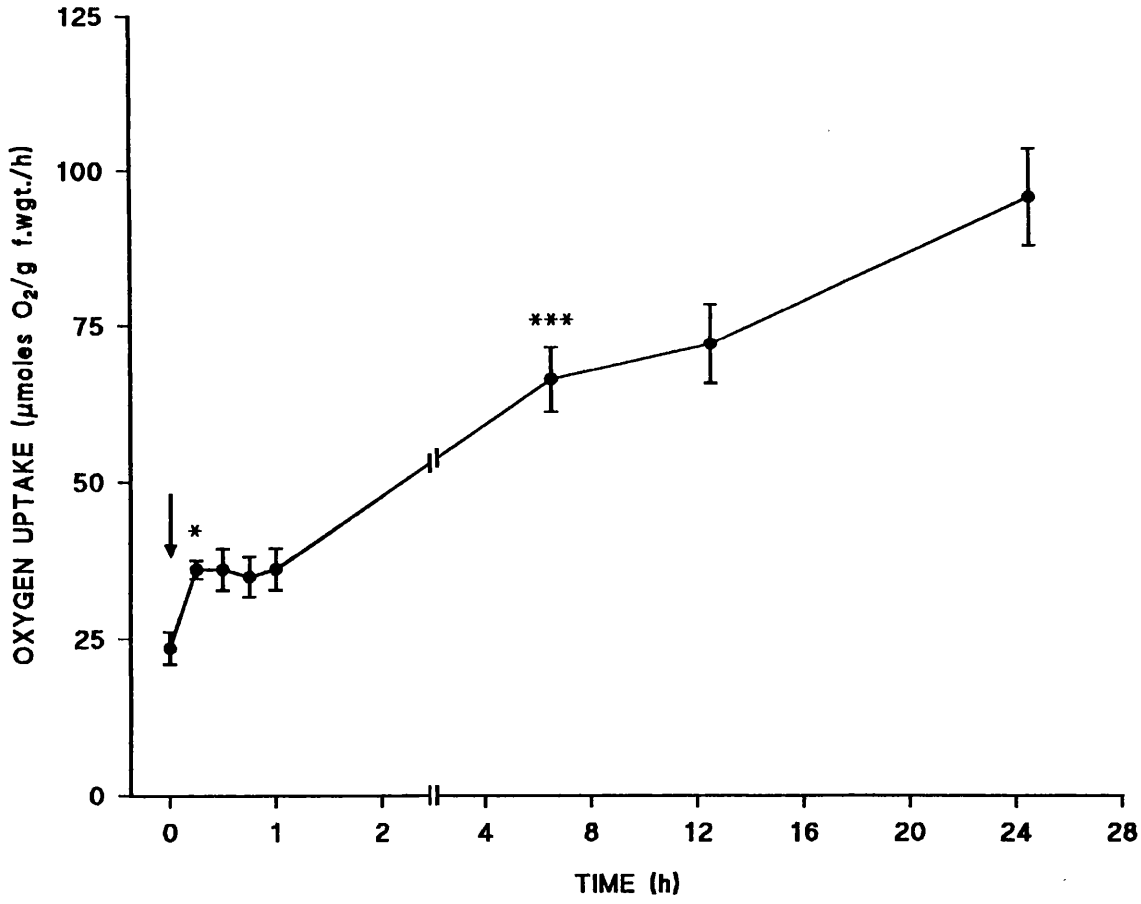
Seeds were either ■, imbibed for 24 h; or ●, conditioned for 7 days before measurement of O_2 uptake for 1 h at the times indicated. Arrow indicates addition of ACC to 50mM. Error bars indicate ± S.E.; stars indicate a significant difference from the rate prior to addition of ACC; * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $P \leq 0.001$, N.S. = not significant.

Figure 3.32

Effect of ethylene on the O_2 uptake of *Striga hermonthica* seeds.

Seeds were conditioned for 7 days before measurement of O_2 uptake for 1 h at the times indicated as detailed in 2.8. Arrow indicates the addition of ethylene. Error bars indicate ± S.E.; stars indicate a significant difference from the rate prior to addition of ethylene; * = $0.05 \geq P > 0.01$, ** = $0.01 \geq P > 0.001$, *** = $P \leq 0.001$, N.S. = not significant.


150
100
50
25
0
OXYGEN UPTAKE (µmoles O_2/g f.wgt./h)
***
**
***
0
1
2
4
8
12
16
20
24
28
TIME (h)


125
100
75
50
25
0
OXYGEN UPTAKE (µmoles O_2/g f.wgt./h)
*
***
0
1
2
4
8
12
16
20
24
28
TIME (h)

day conditioned seed as discussed for ACC above, the initial increase is greater, with ethylene treated seeds respiring at 36.1 ± 1.4 μmoles h^{-1} g^{-1} over the first 15 min relative to a basal level of 23.5 ± 2.5 μmoles h^{-1} g^{-1} before addition of ethylene (Fig. 3.32). Although the rates after 6 h stimulation with either ethylene or ACC are not significantly different, treatment with ethylene does not result in a rapid rate increase between 6 and 12 h as measured with seed stimulated by ACC.

Incubation of 7 day conditioned seed with thidiazuron results in a pattern of O_2 uptake similar to that observed with GR-24 treatment (*cf.* Fig. 3.29 and 3.30). Addition of thidiazuron results in an immediate slight increase in the rate of O_2 uptake which continues to rise steadily over the next 12 h. Between 12 and 24 h the rate of O_2 uptake increases again (*cf.* GR-24 treatment, Fig. 3.29) although the rate at 24 h is significantly lower than that obtained following incubation with GR-24. After 24 h incubation in thidiazuron, respiration rates have reached the levels of ACC stimulated seed while being significantly higher than that observed with ethylene treatment.

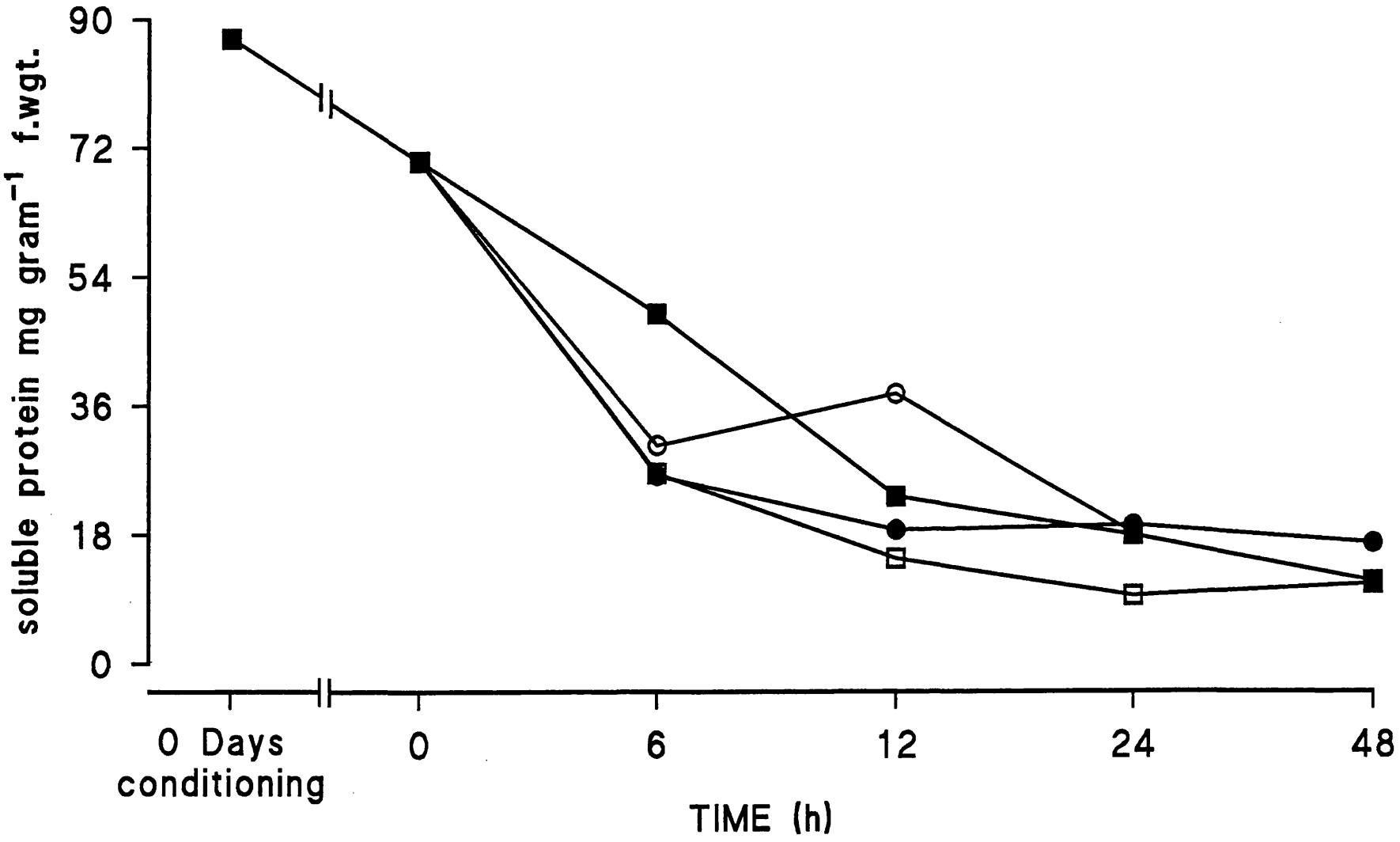
3.4 Protein and Amino Acids

3.4.1. Soluble protein and amino acid content

The amount of water soluble protein was measured, before and after conditioning and at times during incubation in GR-24, thidiazuron, ACC and ethylene (Fig. 3.33). During the conditioning period there is a drop in soluble protein content which may be attributable to the hydrolysis of soluble reserve proteins in order to provide amino acids for the synthesis of non-water soluble

Figure 3.33

Change in the water soluble protein content of seeds of *Striga hermonthica* during conditioning and after incubation in: ■, 3.4μM GR-24; □, 32μL/L ethylene; ●, 50mM ACC; or ○, 1μM thidiazuron. Time zero = 7 days conditioning.


soluble protein mg gram^{-1} f.wgt.
90
72
54
36
18
0
0 Days
conditioning
0
6
12
24
48
TIME (h)

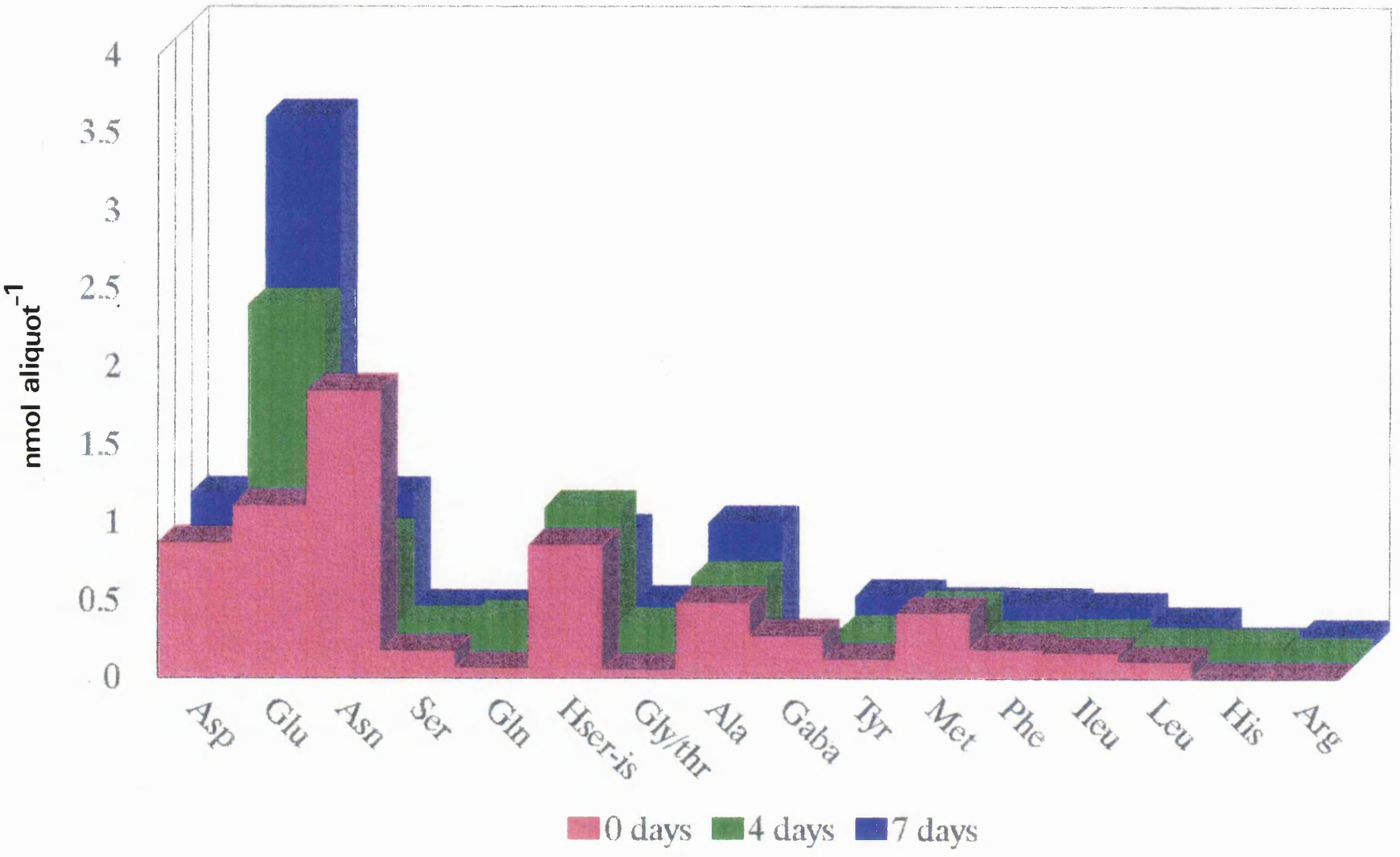
proteins, alternatively soluble protein may be used as a respiratory substrate. Incubation with any of the germination stimulants results in a similar pattern of depletion of water soluble proteins (Fig. 3.33). Following addition of thidiazuron, ACC or ethylene at time zero there is a rapid decrease in soluble protein content over the first 6 h which levels off between 6 h and 48 h, treatment with GR-24 does not cause as rapid a decrease.

In seeds of species with non-water-soluble protein reserves there is frequently an increase in the soluble protein content attributable to the hydrolysis of these reserves at the onset of germination. If hydrolysis of insoluble reserves to soluble products is part of the germination process in *Striga*, then it occurs at a level much lower than the synthesis of insoluble protein from soluble protein derived amino acids or the respiration of soluble protein.

The free amino acid content of *S. hermonthica* seeds increases during the conditioning process, probably due to the hydrolysis of protein reserves (Fig. 3.34). The majority of this hydrolysis occurs during 0 to 4 days conditioning when there is a significant rise in the amounts of free glutamic acid, glutamine, glycine/threonine ($0.01 \geq P > 0.001$) and tyrosine ($0.05 \geq P > 0.01$). In addition, histidine and arginine are detected and there is a significant decrease in the amount of asparagine ($0.01 \geq P > 0.001$). Between 4 and 7 days conditioning there is an increase in the amounts of free alanine ($0.05 \geq P > 0.01$) and isoleucine ($0.01 \geq P > 0.001$) while there is a significant decrease in the amount of histidine ($0.05 \geq P > 0.01$).

Figure 3.34

Amino acid content of *Striga hermonthica* seeds after conditioning for 0, 4 or 7 days.


nmol aliquot-1
4
3.5
3
2.5
2
1.5
1
0.5
0
Asp
Glu
Asn
Ser
Gln
Hser-is
Gly/thr
Ala
Gaba
Tyr
Met
Phe
Ileu
Leu
His
Arg
0 days
4 days
7 days

3.4.2 1-D SDS-PAGE of conditioned seed

The resolution of proteins by gel electrophoresis allows the disappearance of polypeptides present in the dry seed to be qualified together with the appearance of new polypeptides formed by *de novo* synthesis, or by the break-down of other proteins. Analysis of soluble protein from *S. hermonthica* seeds during conditioning by 1-D SDS-PAGE and gel scanning reveals little change in the polypeptide pattern (Fig.3.35). Some differences are apparent following hydration (compare 0 and 6 days conditioning, arrows) but subsequently, between 6 and 26 days conditioning, there are no significant qualitative changes in protein bands.

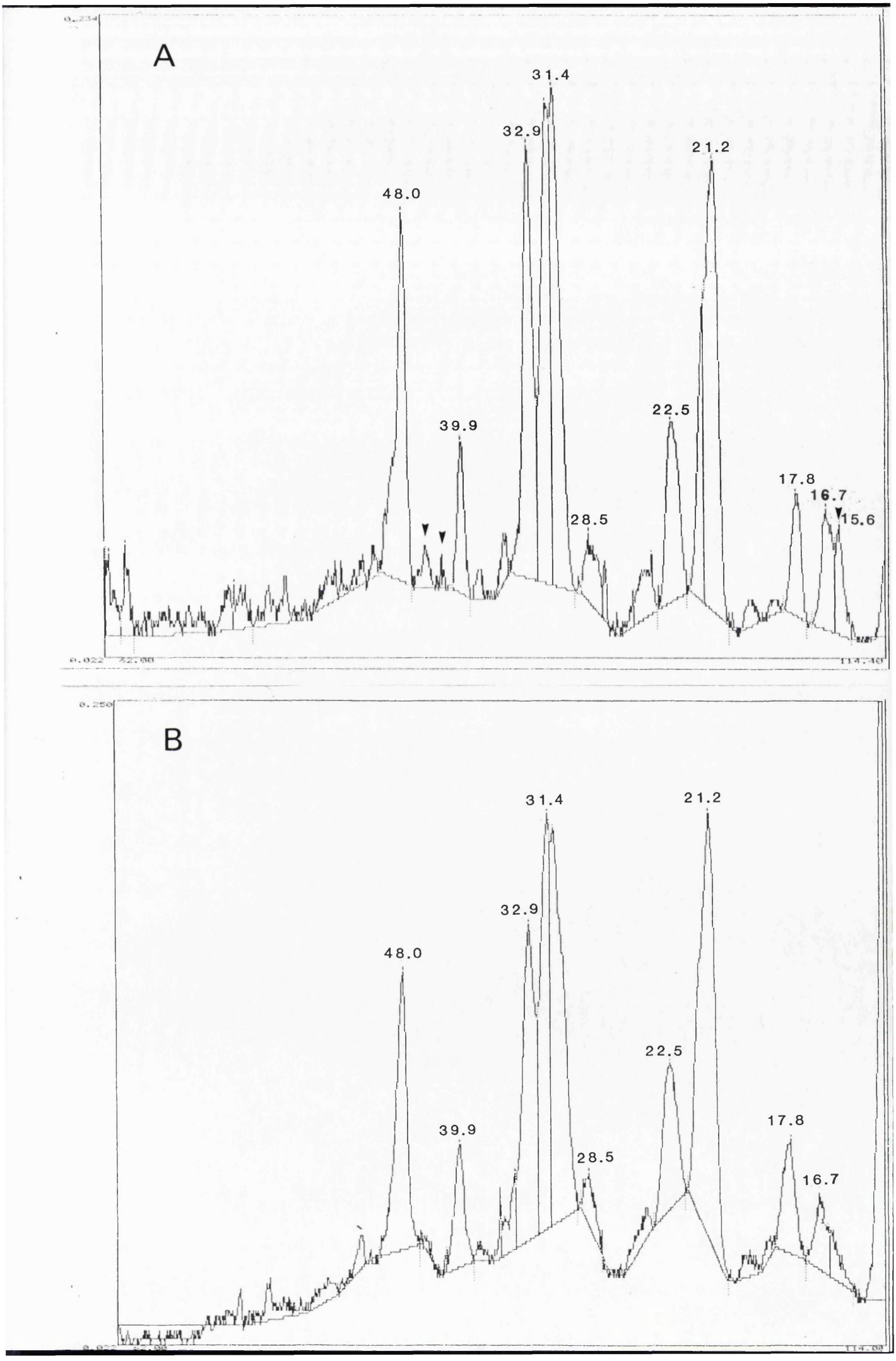
3.4.3 2-D PAGE of conditioned and germinating seed

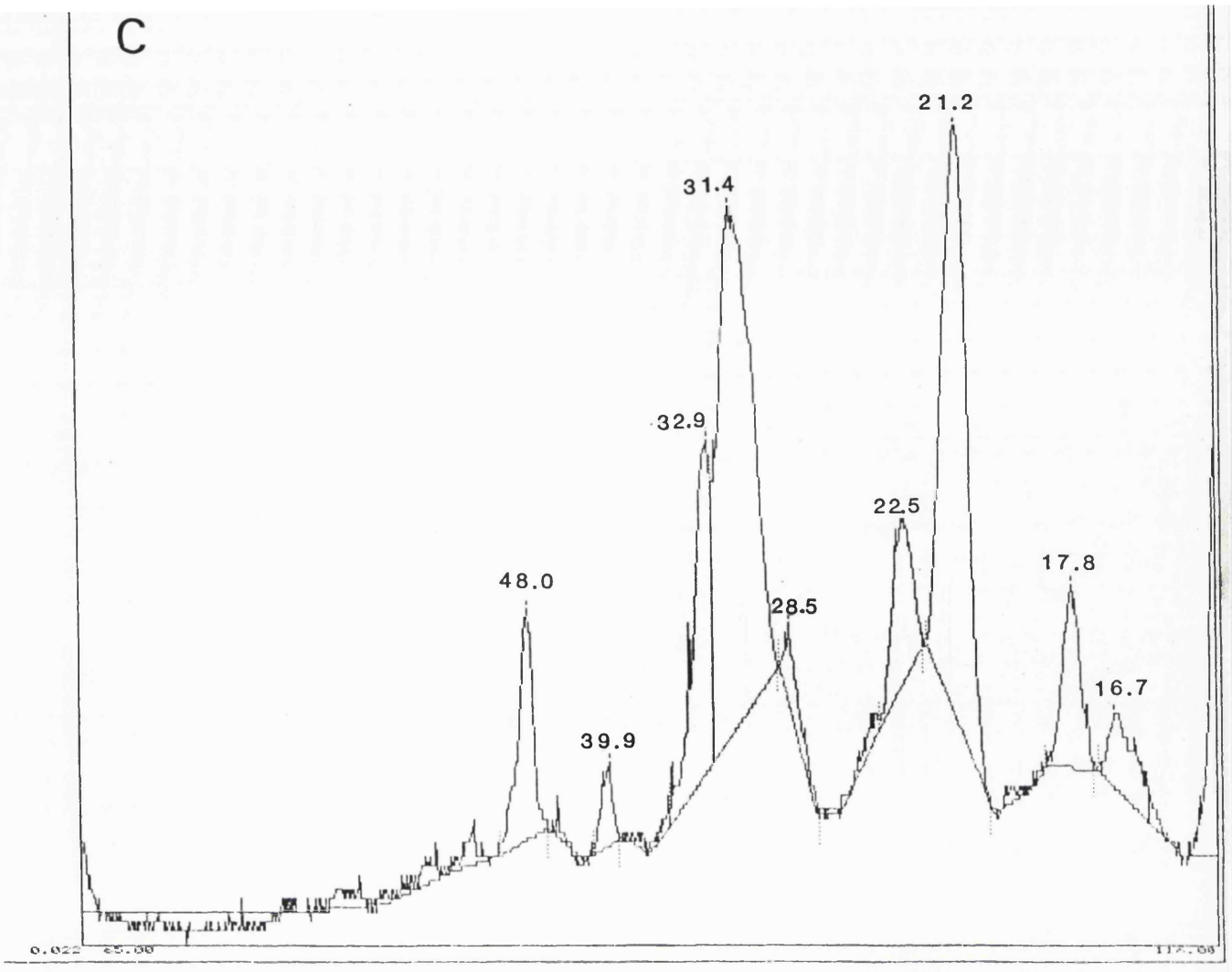
Comparison by 2-D SDS-PAGE provides greater resolution allowing qualitative changes of polypeptides that are not apparent in 1-D SDS-PAGE to be detected. When soluble protein extracts from conditioned *S. hermonthica* seed were analyzed, many changes in the water-soluble protein composition were observed (Fig.3.37). Comparison of polypeptide patterns from 0 and 7 day conditioned seed reveals a significant change in protein pattern during conditioning; there are more polypeptides present at 0 days with higher molecular weight proteins present ($> M_r = 46,000$).

In order to determine which proteins present arise from *de novo* protein synthesis during conditioning *in vivo* labelling, with ^{35}S-methionine, was carried out. It was not possible to visualise *in vivo* labelled proteins during the onset of imbibition (0 - 2 h), presumably as the tissue has not completely rehydrated, thus

Figure 3.35

Densitometric scans of 1D SDS-PAGE gels of *Striga hermonthica* seed soluble protein. Seed was conditioned for **A.** 0 days; **B.** 6 days; **C.** 16 days; **D.** 26 days.


A
31.4
32.9
21.2
48.0
22.5
39.9
17.8
16.7
28.5
15.6
B
31.4
21.2
32.9
48.0
22.5
39.9
17.8
28.5
16.7


C
21.2
31.4
32.9
22.5
48.0
28.5
17.8
16.7
39.9

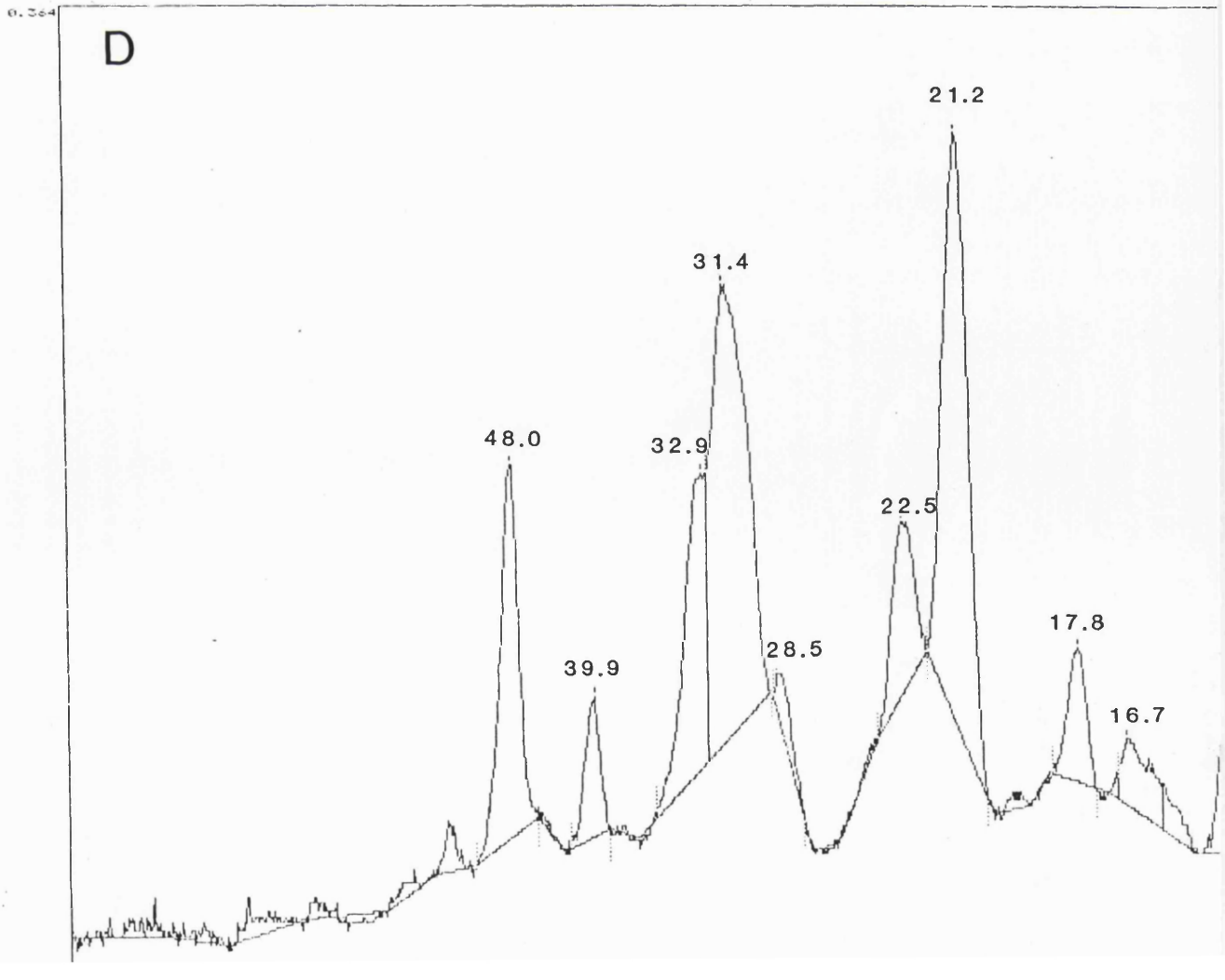
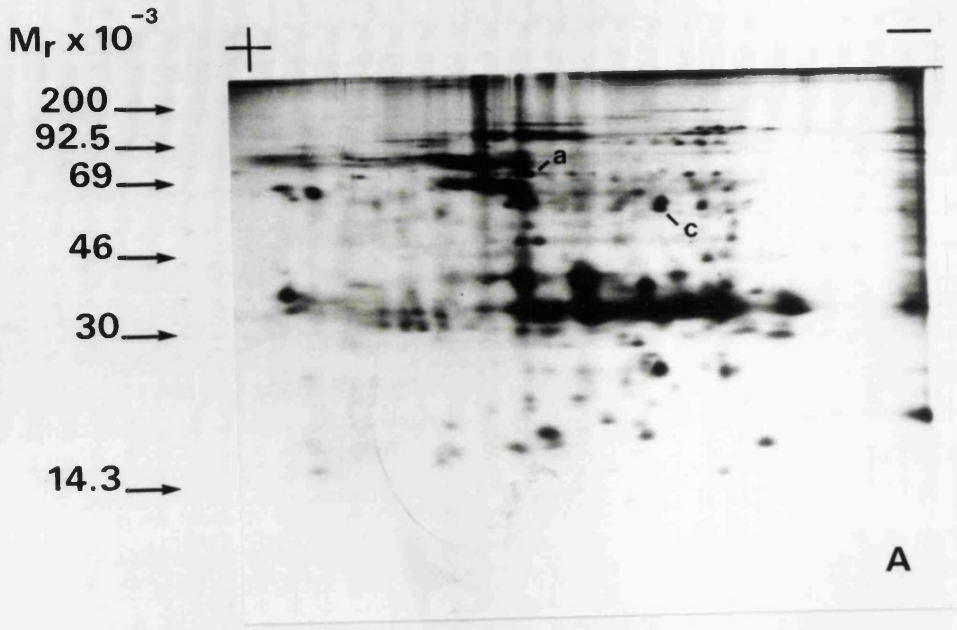

D
21.2
31.4
48.0
32.9
22.5
17.8
28.5
39.9
16.7

Figure 3.36

2-D SDS-PAGE of soluble protein in 24 h imbibed *Striga hermonthica* seed. **A.** Silver stained gel and **B.** ^{35}S-labelled fluorograph. Labelling was carried out as detailed in 2.6.5.


$M_r \times 10^{-3}$
200
92.5
69
46
30
14.3
+
—
a
c
A

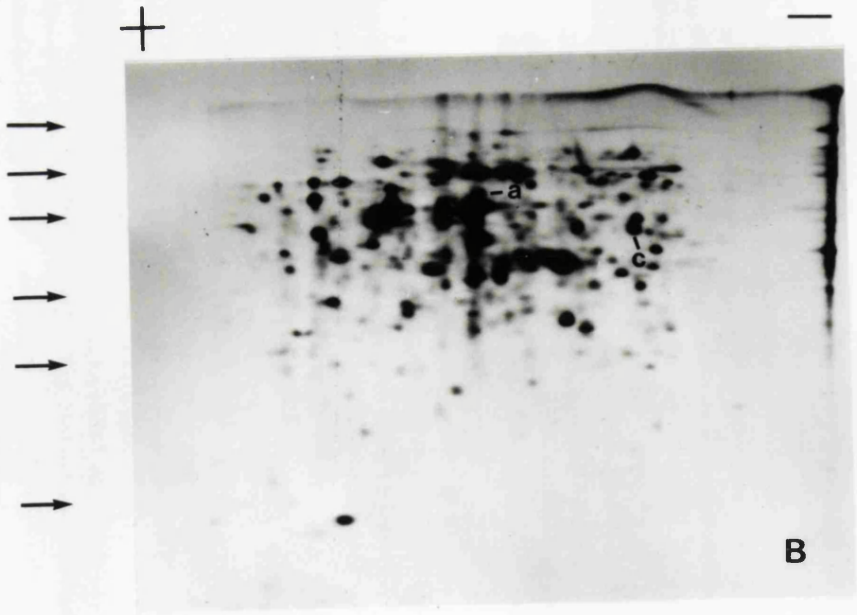
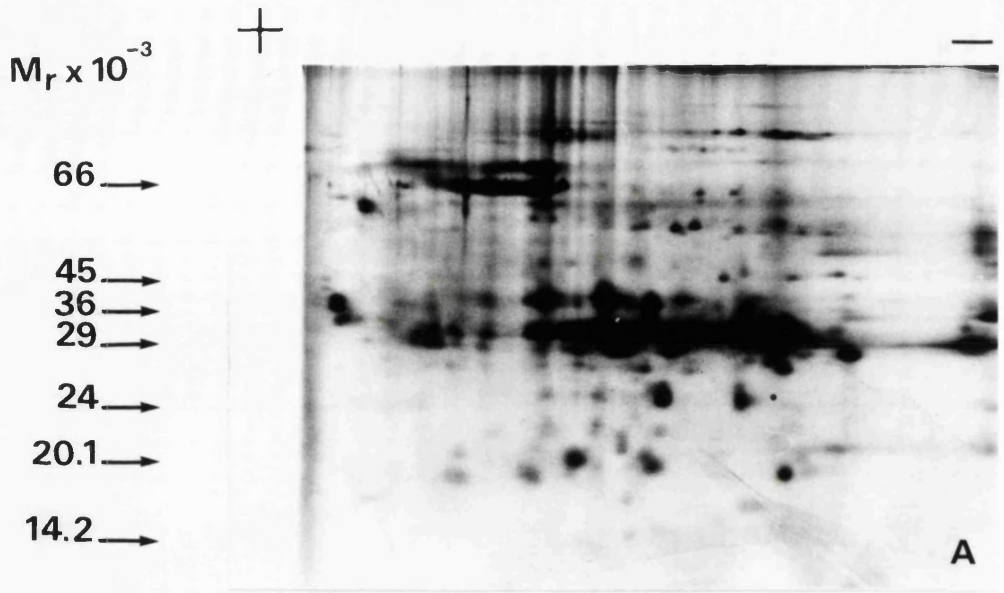

+
—
a
c
B

Figure 3.37

Silver stained 2D SDS-PAGE gels of soluble protein in *Striga hermonthica*. **A.** 0 days conditioning, **B.** 7 days conditioning.


$M_r \times 10^{-3}$
66
45
36
29
24
20.1
14.2
A

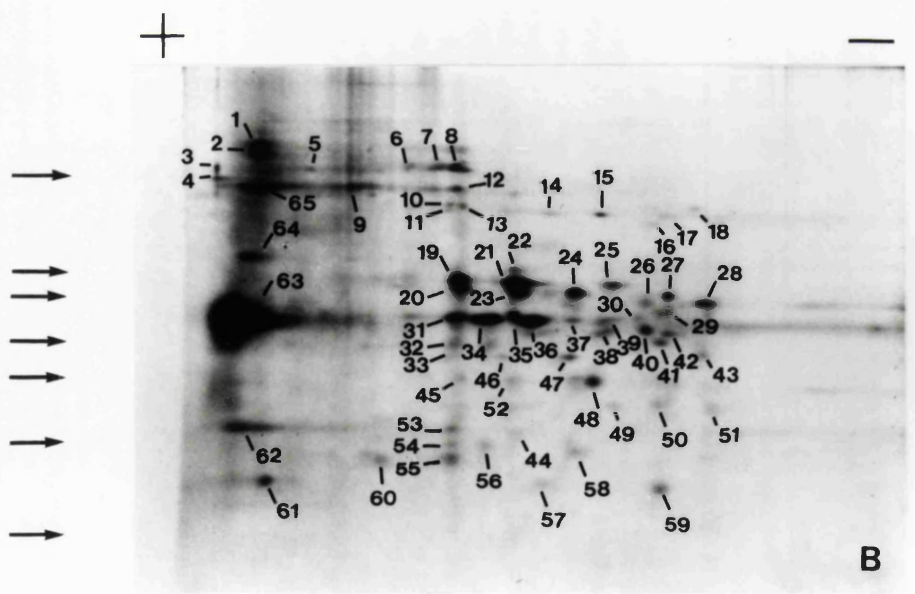

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
B

Figure 3.38

Silver stained 2D SDS-PAGE gels of soluble protein in *Striga hermonthica* seeds incubated in 3.4μM GR-24.

Seeds were conditioned for 7 days before transfer to 3.4μM GR-24 for: **A.** 6 h; **B.** 12 h; **C.** 24 h; **D.** 48 h.

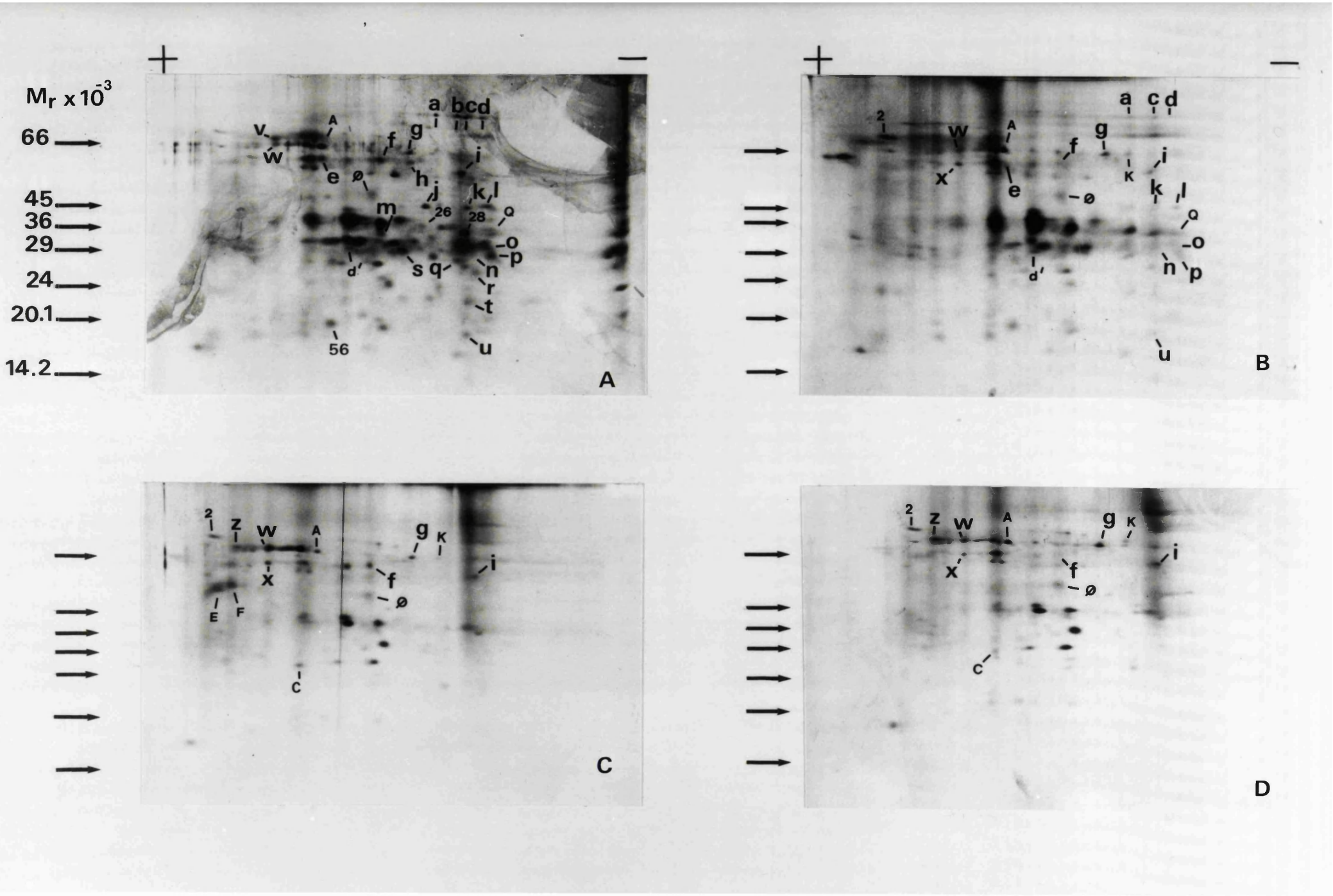

Mr x 10-3
66
45
36
29
24
20.1
14.2
+
−
A
B
C
D

Figure 3.39

Silver stained 2D SDS-PAGE gels of soluble protein in *Striga hermonthica* seeds incubated in 1μM thidiazuron.

Seeds were conditioned for 7 days before transfer to 1μM thidiazuron for: **A**. 6 h; **B**. 12 h; **C**. 24 h; **D**. 48 h.

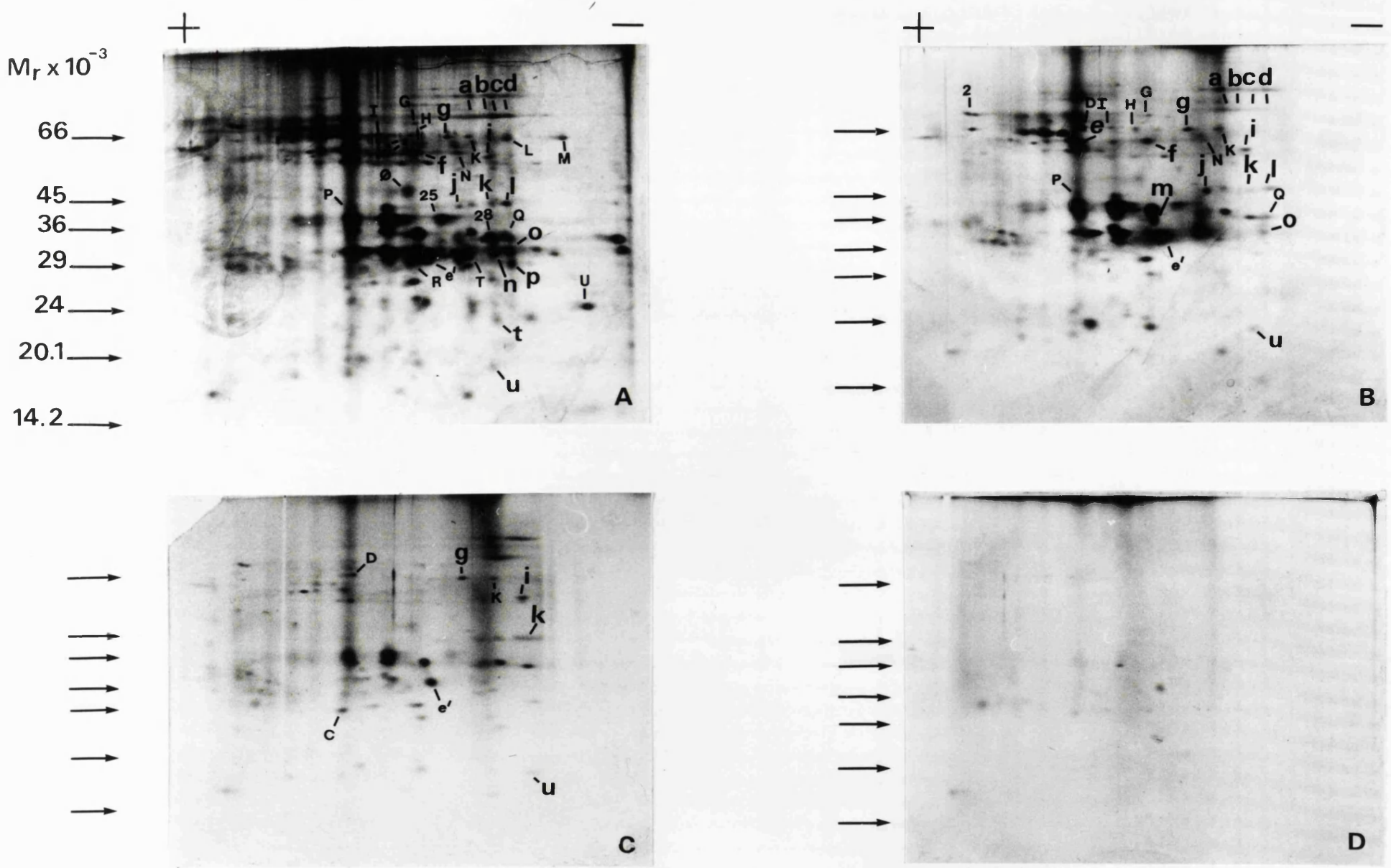

Mr x 10^-3
66
45
36
29
24
20.1
14.2
+
−
abcd
A
B
C
D

Figure 3.40

Silver stained 2D SDS-PAGE gels of soluble protein in *Striga hermonthica* seeds incubated in 50mM ACC.

Seeds were conditioned for 7 days before transfer to 50mM ACC for: **A**. 6 h; **B**. 12 h; **C**. 24 h; **D**. 48 h.

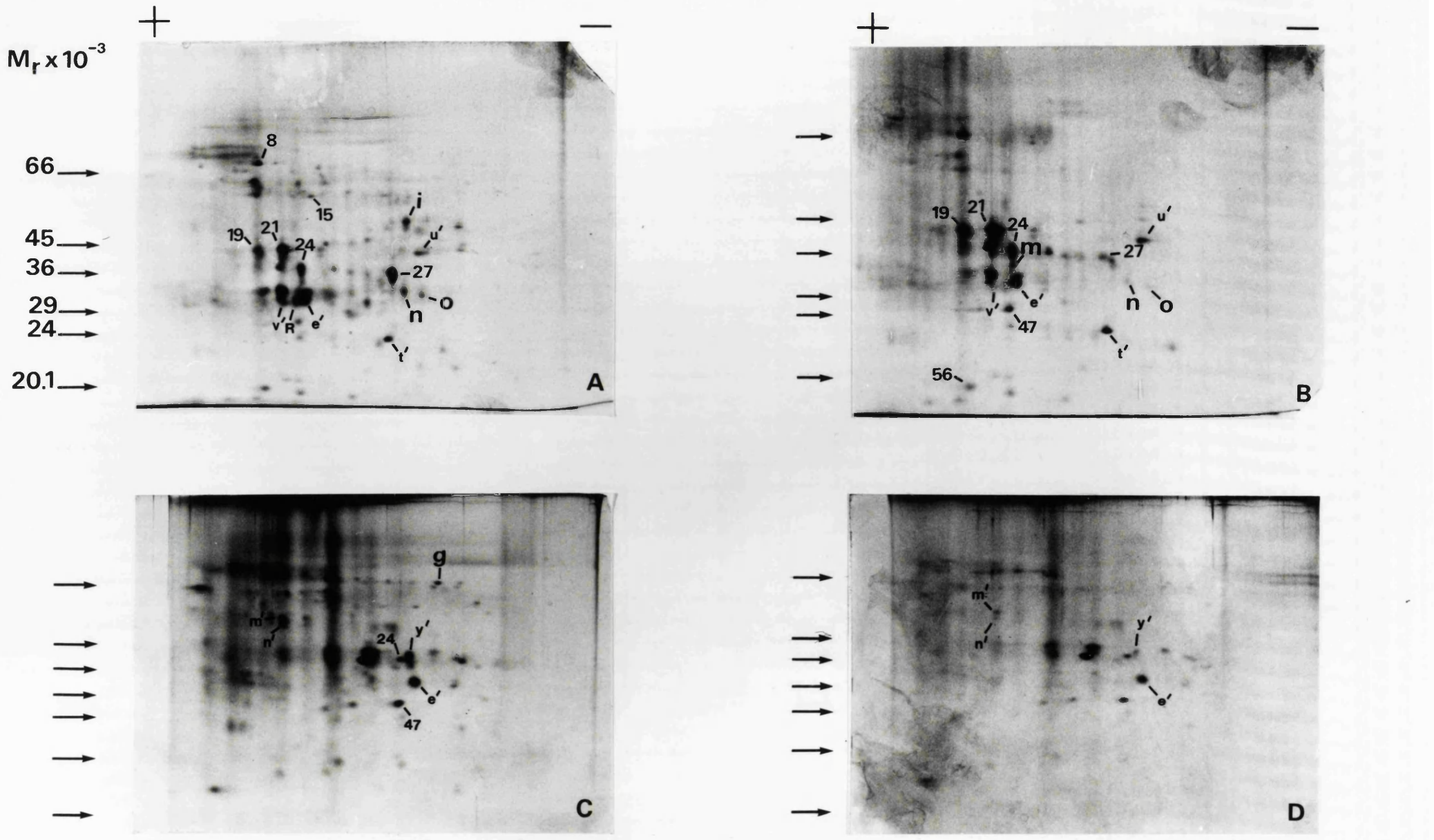
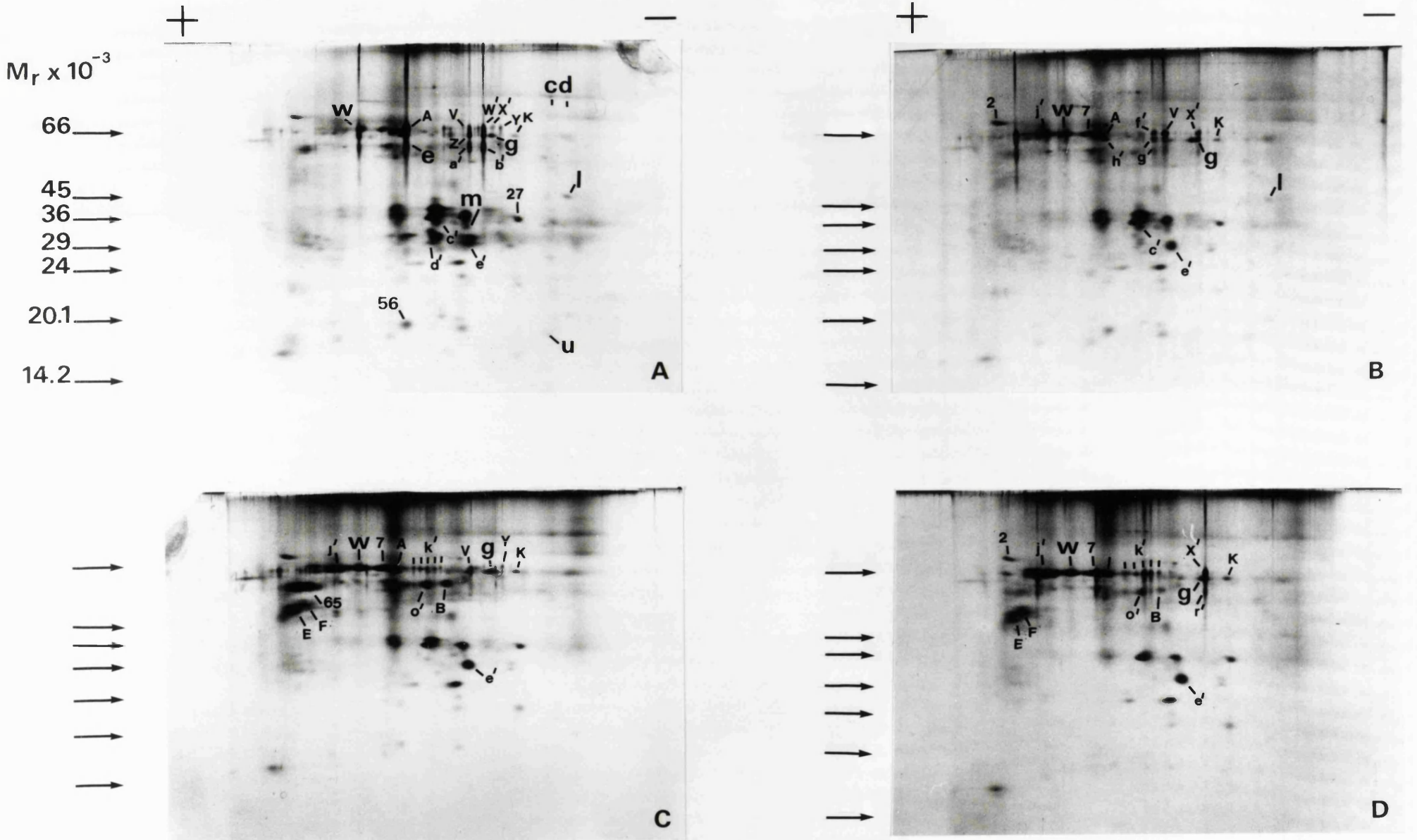

$M_r \times 10^{-3}$
66
45
36
29
24
20.1
+
−
8
15
i
19
21
24
u′
27
o
n
v′
R
e′
t′
A
m
56
47
B
g
m′
n′
y′
C
D

Figure 3.41

Silver stained 2D SDS-PAGE gels of soluble protein in *Striga hermonthica* seeds incubated in 32μL/L ethylene.

Seeds were conditioned for 7 days before transfer to 32μL/L ethylene for: **A**. 6 h; **B**. 12 h; **C**. 24 h; **D**. 48 h.


+
−
$M_r \times 10^{-3}$
66
45
36
29
24
20.1
14.2
w
A
V
W X′
Y K
cd
e
z
a′
b
g
l
m
27
c′
d′
e′
56
u
A
2
j′
W
7
A
f′
V
X′
K
h′
g′
g
l
c′
e′
B
j′
W
7
A
k′
V
g
Y
K
65
E
F
o′
B
e′
C
2
j′
W
7
k′
X′
K
g
r′
o′
B
E
F
e′
D

limiting uptake of label. Increasing the conditioning time from 24 h to 4 or 7 days results in a greater rate of radiolabel incorporation into protein with each increase in conditioning time (Table 3.16). This increase in the rate of radiolabel incorporation could result from a variety of mechanisms. Uptake into the seed may become greater as the tissue becomes more permeable during hydrolysis but while this may explain the initial changes, after 4 and 7 days complete imbibition would be expected. The increase in radiolabel uptake could be due to increased protein synthesis or a greater rate of protein turnover ie. degradation and *de novo* synthesis. *In vivo* labelling of seed conditioned for 24 h reveals the synthesis of many proteins, the majority of which have an apparent molecular weight greater than M_r = 46,000 (Fig. 3.36).

Silver-stained 2-D SDS-PAGE gels were used to analyze differences in polypeptide patterns during conditioning and germination in the presence of different germination stimulants (Fig 3.37, 3.38, 3.39, 3.40, 3.41). The major water-soluble polypeptides present in 7 day conditioned seed are labelled in Fig. 3.37 with a number while those spots which are newly detected at each treatment time (Fig. 3.38, 3.39, 3.40, 3.41) are labelled with a letter (either lower case, upper case, or lower case prime). Spots present at 7 days are not labelled at later stages in the time-course, except where used as positional markers. Polypeptide spots newly detected in treatments with GR-24, thidiazuron, ACC or ethylene are labelled when initially observed and at subsequent stages where their presence remains indisputable. In order to simplify comparisons of these polypeptide changes they are collated in Fig. 3.42.

Analysis of the polypeptide pattern of seed stimulated by GR-24,

Table 3.16

Incorporation of ^{35}S-methionine into TCA precipitable protein.

Conditioning time (days)	cpm
0	267
1	4341
4	7958
7	10668

cpm - counts per minute obtained with TCA precipitated aliquot of homogenised sample prepared as detailed in 2.6.5.

Figure 3.42

Comparison of polypeptide patterns obtained from 2D SDS-PAGE gels shown in Figures 3.38 - 3.41.

2-DIMENTIONAL PAGE ANALYSIS

TREATMENT	DISAPPEARED
6 h.	
GR-24	1 11 29 44 49 63 64 65
THIDIAZURON	1 29 59 64 65
ETHYLENE	1 10 14 15 16 17 28 29 32 33 38 39 42 48 49 51 52 53 54 59 63 64
ACC	1 2 3 4 6 7 9 10 11 16 17 18 20 22 25 26 28 29 31 32 33 36 37 38 41 42 43 45 46 48 49 52 53 54 58 63 64 65
12 h.	
GR-24	3 4 16 17 26 30 38 40 48 49 b e° f h j m q r s v
THIDIAZURON	3 5 11 16 17 18 33 38 39 45 t A J L M R T U Øn p
ETHYLENE	22 30 31 34 40 42 43 57 59 62 65 Z a' b' W'
ACC	5 30 34 40 50 v'°
24 h.	
GR-24	15 22 25 29 31 32 33 34 35 36 37 39 42 51 52 53 57 60 k l n o p u d'
THIDIAZURON	4 6 7 8 14 15 22 25 30 31 34 37 40 43 50 51 53 54 57 e f j l m G° H I N O
ETHYLENE	25 26 28 36 44 c' f' g' X'
ACC	34 35 51 57 62 m n o t' u' v'

48 h.

GR-24 f E F

THIDIAZURON

ETHYLENE 19 21 22 25 35 45 55 56

ACC 12 13 27 55 56 60 c d g° n'

TREATMENT	NEW
6 h.	
GR-24	a b c d e f g h i j k l m n o p q r s u v w A Ø
THIDIAZURON	a b c d e f g i j k l n o p t u v w A G H I J K L M N P Q R TØ
ETHYLENE	c d e g l m u w A K V Y Z a' b' c' d' e' W' X'
ACC	i n o R e' t' u' v'
12 h.	
GR-24	59 K X
THIDIAZURON	m D
ETHYLENE	f' g' h' j'
ACC	m
24 h.	
GR-24	C E F
THIDIAZURON	C
ETHYLENE	65 B E F k' o' y'
ACC	14 15 m' n' y'

thidiazuron, ACC or ethylene reveals similarities between GR-24 and thidiazuron treatments (Figs. 3.38 and 3.39) and between ACC and ethylene treatments (Figs. 3.40 and 3.41). Polypeptides associated with the germination process (ethylene) and not with the perception and transduction of the host derived germination stimulant (GR-24) are apparent from the comparison of GR-24 and ethylene treatments. Many spots disappear after 6 h treatment with ethylene which are still present 6 h after GR-24, however, this is only a temporal dissimilarity. The majority of these spots disappear later with GR-24 treatment (eg. spots 15, 16, 17, 32, 33, 38, 39, 42, 48, 49, 51, 52, 53). Stimulation of germination by GR-24 or thidiazuron results in spots disappearing at the same time, notably at 12 h and 24 h (spots 3, 16, 17, 38; 15, 22, 31, 37, 53, l, respectively).

There are also parallels in the appearance of new (= previously undetected) polypeptides in seed stimulated by GR-24 and thidiazuron some of which appear in ACC but not ethylene treated seed. Numerous polypeptide spots are detectable only after stimulation, many being specific to one treatment only (thidiazuron in particular). However, of the 23 new spots after 6 h with GR-24 only 4 are unique to this treatment (h, q, r, s). Spots common to GR-24 and thidiazuron but not present in ethylene or ACC stimulated seed are not involved directly with germination and may result from changes within the seed specific to the mode of action of GR-24 and thidiazuron in triggering germination i.e. stimulation of ethylene production (a, b, f, j, k, p, C).

The many new polypeptides unique to thidiazuron treatment (Fig. 3.39), unlabelled because of the number, may result from the cytokinin activity of this compound and not from the stimulation of ethylene production. Many

polypeptides disappear during the first 6 h incubation in ACC, although some also disappear in ethylene treated seed, the majority of these polypeptides are also lost at a later time from seeds stimulated by the other treatments (except spots 2, 3, 9, 20, 41, 46, 58; Fig. 3.42). It is surprising that there is not a greater parallel between ethylene and ACC treatments. Although there is a degree of similarity when comparing the disappearance of polypeptides, there are only two new polypeptides common to both treatments (e', f').

Those spots which are omnipresent in all treatments are likely to function in vital "house-keeping" roles of primary metabolism (19, 23, 24, 47, 61).

CHAPTER 4 - DISCUSSION

A seed is a propagule and for germination to result in successful seedling establishment germination must occur when environmental conditions favour seedling growth and vigour. Many strategies have evolved within the Angiospermae which act to increase the likelihood of germination occurring under optimal or near-optimal conditions. Common among these strategies are those involving some form of dormancy. Postponement of germination following seed shed, until such time as conditions are favourable, may confer an advantage over non-dormant seeds; germination of the annual seed population of many species is spread over a number of years resulting in a soil seed bank containing different generations of seed (Egley, 1984; Bouwmeester and Karssen, 1989).

In order to survive, obligate parasitic plants require a suitable host from which they can obtain water and photosynthates. It is essential that seed germination of such parasite species is synchronised to the presence of a host. This could be achieved if a long period of imbibition (i.e. period of suitable soil moisture) was required by the parasitic seed, relative to the host, prior to germination. The requirement for a prolonged period of imbibition, known as the conditioning period, prior to stimulation of germination by host derived chemicals is found in many species of root-parasitic angiosperms (see 1.1.1.3). It is possible that this imbibition period increases the chance of survival for the parasitic seedling, by ensuring establishment or continued vigour of a host.

The results of Vallance (1950, 1951a,b) demonstrated the conditioning process to be an absolute prerequisite for host stimulated germination in *Striga*

hermonthica, and that the duration of this process is dependant upon seed age; older seed requiring a shorter conditioning period than younger seed. Such a conclusion cannot be made with confidence from the results presented in Figs. 3.1, 3.2 and 3.3; there is no apparent relationship between length of conditioning period and percentage germination. It is possible, however, that any true effect of increased conditioning time or increased seed age is masked by variability in the concentration/composition of host root exudate (see below).

Vallance's findings are in agreement with those of other workers, using *S. asiatica*, who found that optimum duration and temperature for conditioning varied between seed vintages. However, as with the results presented in the preceding chapter (Figs. 3.2 and 3.3), germination was erratic (Brown and Edwards, 1946; Kust, 1963; Pavlista *et al.*, 1979; Reid and Parker, 1979). The erratic germination response to host root exudate may confer a survival advantage to *Striga*, such that germination of each seed generation is spread over a number of years, as is common in many species of non-parasitic angiosperm. Recently, workers from the University of Nijmegen in the Netherlands observed that the percentage of *S. hermonthica* seeds stimulated to germinate by GR-24 varied cyclically with time of year (Mangus *et al.*, 1992) a result which adds to the already complicated germination behaviour of this parasite.

The rate of oxygen uptake by *S. hermonthica* seeds during conditioning shows a significant peak after 2 or 6 days for 9 or 1 year old seed samples respectively (Figs. 3.27 and 3.28), however, variability of the germination response to host root exudate precludes any demonstration of a correlation between respiration rate and germination percentage. Vallance (1951a) concluded

that germination percentage and respiration rates during conditioning were linked, although seed of different ages had markedly different correlations between conditioning time, respiration rate and germination. The work of Vallance showed that while 6 year old seed (styled "overripe") showed a cyclical respiration pattern, the respiration rate of 2 year old seed (styled "immature") declined as the conditioning time increased from 3 to 33 days. No such differences in rate of O_2 uptake is seen for 1 and 9 year old seed in this study (Figs. 3.27 and 3.28).

A possible physiological explanation for the conditioning process is the requirement for the mobilisation of stored reserves. Mobilisation of protein reserves results in the breakdown of proteins to amino acids and lower molecular weight proteins. Analysis of the change in free amino acid content of *S. hermonthica* seed reveals significant increases in those amino acids which serve as organic nitrogen sources (glutamine and glutamic acid) along with significant increases in several other amino acids (see 3.4.1 and Fig. 3.34). It is likely that these increases result from the hydrolysis of protein reserves to provide a pool of amino acids for protein synthesis. However, since it is not known how the germination process affects the free amino acid content of *Striga* seeds, it may be that the majority of protein hydrolysis occurs during germination as in non-parasitic seeds. Analysis of the change in polypeptide composition of *S. hermonthica* by 2D SDS-PAGE reveals an overall decrease in soluble protein with a reduction in the amount of higher molecular weight polypeptides during conditioning (0 to 7 days) (Fig. 3.37). Direct measurement of the soluble protein of *S. hermonthica* confirms the reduction in soluble protein content during

conditioning (Fig. 3.33). While the reduction in higher weight polypeptides appears no greater than the reduction in most of the polypeptides present in the dry seed, the significant changes in the free amino acid content suggests that mobilisation of reserves may partly explain the requirement for the conditioning process, although any mobilization after 24 h imbibition is not required for germination in response to ethylene, ACC and thidiazuron (Fig. 3.21). Menetrez (1986) presented evidence demonstrating that the major storage product in *Striga asiatica* seeds was lipid, with an average lipid content of 37.5% of seed dry weight, equivalent to that of rape seeds. However, there was not a decline in the total levels of lipid in germinating *S. asiatica* seeds as found for other oil seeds (Bewley and Black, 1983). This may be due to the presence of a combination of stored reserves in *Striga* each of which is utilized at a particular phase of the conditioning/germination process. Since *Striga* seeds may go through the conditioning process several times before stimulation of germination, it would be advantageous to preserve some energy sources specifically for germination.

Various other hypotheses have been suggested to account for the conditioning process. Germination inhibitors present in *Striga* (Williams, 1959; Kust, 1966) could ensure dormancy during an environmentally unsuitable period; leaching of a soluble inhibitor from the seeds during conditioning could release the seed from its dormant state. Changes occur within the seed during prolonged imbibition (conditioning) which confer the ability to germinate in response to both natural host derived chemicals (Table 3.11) and to the synthetic strigol analogue GR-24 (Fig 3.17). These internal changes are not necessary for the germination

process *per se*, as exemplified by the ability of exogenous ethylene or ACC to greatly stimulate germination of imbibed but unconditioned seed (Fig. 3.21). This conclusion is corroborated by the respiration data (Fig. 3.29). While stimulation by GR-24 of seed conditioned for 7 days results in a significant increase in O_2 uptake within 1 h and leads to a respiration rate of 261.5 $\pm$ 33.1 μmoles h^{-1} g^{-1}, 23 h later, treatment of 24 h imbibed seed results in no significant change to the respiration rate 24 h later. These results suggest that there is a block or lesion in the natural germination pathway which is overcome by conditioning.

Brown and Edwards (1946) proposed that the *Striga* seed produces a stimulating substance "which is the same or similar to that which originates from the host root" and that the level of this substance first increases then decreases during the conditioning process. They suggested that the external stimulus, host root exudate, acts together with the internal stimulant concentration to increase the stimulant concentration above a minimum required for initiation of germination. In contrast to this view, the evidence presented in this thesis demonstrates the involvement of ethylene as the endogenous stimulant.

Ethylene is produced by germinating seeds of many plant species, and endogenous ethylene has been shown to stimulate germination of *Lactuca sativa* L., *Xanthium spp.*, *Cucumis anguria* L., *Arachis hypogaea* L., *Amaranthus caudatus* L. and *Chenopodium album* L. (Abeles, 1986; Katoh and Esashi, 1975a,b; Ketring and Morgan, 1972; Kepczynski and Karssen, 1985; Machabee and Saini, 1991). The ability of the ethylene biosynthesis inhibitor AVG to completely inhibit host stimulated germination provides the first evidence for the role of endogenous ethylene in the stimulation of germination in *S. hermonthica*

(Fig. 3.8). Confirmation that this reduction in germination is a result of inhibiting ethylene biosynthesis is provided by the rapid production of ethylene following incubation with host root exudate (Fig. 3.23) and its concomitant inhibition by the inclusion of AVG in the incubation medium (Table 3.13). AVG has been used in several studies on non-parasitic angiosperms (*Lactuca sativa, Chenopodium album* and *Amaranthus caudatus*) to assess the role of endogenous ethylene in seed germination, unfortunately, in these studies no data was presented on the concentration dependence of this inhibition so a full comparison with the data shown in Figs 3.8, 3.9, 3.14, 3.15 and Table 3.2 is not possible.

Germination of *Lactuca sativa* seeds is strongly influenced by temperature; temperatures above a certain optimum cause a decline in germination percentage, a situation known as thermoinhibition (Reynolds and Thompson, 1971; Thompson *et al.*, 1979). Over a limited temperature range, thermoinhibition can be overcome (ie. germination is stimulated) by treatments (GA, red-light, kinetin, CO_2) which have been shown to be mediated by endogenous ethylene (Saini *et al.*, 1986, 1989). However, although incubating thermoinhibited seed, stimulated by GA, red-light or kinetin, in 1mM AVG causes a massive reduction in ethylene production, down to levels similar to those measured in non-stimulated seed, germination is only reduced by 40-50%. There is no inhibitory effect of AVG on germination of *Amaranthus caudatus* - despite a massive reduction in the amount of ethylene produced by incubation in 1mM AVG the germination of non-dormant seeds is not affected (Kepczynski and Karssen, 1985). A similar result was found with *Chenopodium album* seed (Machabee and Saini, 1991). Using two different seed batches, one possessing a greater percentage of dormant seeds,

Machabee and Saini (1991) found that germination of neither the naturally non-dormant, nor the batch whose dormancy was released by a combination of GA_{4+7}, $NaNO_3$ and light, could be inhibited by 1mM AVG. The lack of a complete inhibitory effect of 1mM AVG on germination of *Amaranthus caudatus, Lactuca sativa* and *Chenopodium album* may result from the minute quantities of ethylene required for germination. Comparison of the amounts of ethylene produced by these seeds with that of *S. hermonthica* is complicated by the fact that the ethylene production results in the studies by Saini *et al.* (1989), Machabee and Saini (1991) and Kepczynski and Karssen (1985) are presented as per 100 or 200 seeds, the weights of which are not given. An approximation can be made using weights from commercial seed catalogues (*Lactuca* - 1000/g; *Amaranthus* - 1750/g, personal communication, J.L.S. Keesing, Royal Botanic Gardens, Kew.). Using these weights the rate of ethylene production by *Lactuca sativa* is 50% of that of *S. hermonthica* when germination was approximately 20%; however, *Amaranthus caudatus* produced only 5% of that of *S. hermonthica*. While it is possible that these differences in ethylene production account for the relative inactivity of AVG as a germination inhibitor, it is more likely that these non-parasitic weeds have a higher sensitivity to ethylene (see below).

As detailed in 1.2, NDE is a competitive inhibitor of ethylene action and has been shown to have a high affinities for a fast-associating ethylene binding site in *Pisum sativum* (Sanders *et al.*, 1991). Inhibition of host root exudate stimulated germination by NDE provides further evidence for the role of ethylene as endogenous germination stimulant in *S. hermonthica*. NDE is less effective an

inhibitor of *S. hermonthica* germination than the ethylene biosynthesis inhibitor AVG, 1mM AVG can completely inhibit host root exudate-stimulated germination although NDE at approximately 20 cm^3/L inhibited germination by between 38% and 89% (cf. Figs. 3.8 and 3.11). As expected with an inhibitor of ethylene action rather than biosynthesis, ethylene production by *S. hermonthica* seeds is not inhibited by NDE - indeed incubation with NDE causes a slight stimulation of ethylene biosynthesis (Table 3.13), which suggests that a negative feedback pathway involving the ethylene (NDE) binding site normally functions to control endogenous ethylene levels, such autoinhibition of ethylene production has been suggested to occur in a number of ethylene producing tissues (Vendrell and McGlasson, 1971; Zeroni *et al.*, 1976; Saltveit and Dilley, 1978). The inhibitory effect of AVG on germination of *S. hermonthica* seed is greater than that observed for those species of non-parasitic angiosperm mentioned above, while NDE appears to inhibit germination of those non-parasitic seeds to a greater degree than it does germination of *S. hermonthica*. Germination of thermoinhibited seeds of *Lactuca sativa* shows a much greater sensitivity to inhibition by NDE than to AVG, incubation in NDE at $2cm^3/L$ reduces germination by over 90% (Saini *et al.*, 1989). A similar result was found for dormant seed of *Chenopodium album*, (NDE at $8cm^3/L$ reduces germination by over 80%) and for non-dormant seed of *Amaranthus caudatus* (NDE at $2.2cm^3/L$ reduces germination from approximately 80% to 0%).

A recent study on the role of ethylene in the germination of *S. hermonthica* and *S. forbesii* found dissimilar effects of GR-24 on ethylene production and action when comparing these two species (Jackson and Parker,

1991). Although ethylene is produced by *S. forbesii*, inhibition of ethylene action by NDE did not lower the subsequent germination percentage. In addition, incubating *S. forbesii* seeds in 1μL/L ethylene failed to stimulate germination. Samples of *S. hermonthica* used by Jackson and Parker (1991) germinated to only 4.8 or 20.6% in response to ethylene although NDE reduced GR-24 germination by 58 or 92% respectively. These results suggest that the link between stimulation of ethylene production and germination is more complex than a simple concentration dependent response.

The seemingly contradictory results of Jackson and Parker (1991), the dissimilarities between the effect of AVG on the germination of those non-parasitic seeds mentioned above and its effect on *S. hermonthica* germination and the effectiveness of NDE as a germination inhibitor of *L. sativa, A. caudatus, and C. album* seed suggest a marked difference in the sensitivities to ethylene both within and between species. There could be an evolutionary advantage to *Striga* if it had a low sensitivity to ethylene, such that germination was not triggered by the background level of ethylene in the soil environment. Germination of obligate parasites such as *S. hermonthica* in response to exogenous ethylene in the absence of a host leads to death within approximately 5 to 6 days.

Root exudates of host plants consist of a complex mixture of chemicals capable of stimulating germination of root parasites (Sunderland, 1960; Visser and Botha, 1974, Visser, 1975; Hauck *et al.*, 1992). Different species of root parasite display a heterogenous response to each particular stimulant with variation in the response of different samples of each parasite species being

apparent. However, the first naturally occurring germination stimulant, strigol, identified from the root exudates of cotton, and the synthetic strigol analogue GR-24, have been shown to stimulate germination of many species of *Striga* and *Orobanche* (Cook *et al.*, 1966, 1972; Kumar and Rangaswamy, 1977; Pavlista *et al.*, 1979). In addition to effects on germination of root parasitic plant seeds, strigol and several synthetic multi-ring analogues have recently been shown to stimulate the germination of dormant seed of both light sensitive and insensitive *L. sativa* to levels significantly greater than controls (Bradow *et al.*, 1988). Indeed, germination percentages obtained with GR-24 and the 2 and 3 ring strigol analogues (2-Ras and 3-Ras) were not significantly different from those obtained under conditions which overcame the endogenous dormancy mechanism (Bradow *et al.*, 1988). Germination of dormant seeds of *A. retroflexus* L. was stimulated approximately 100% by GR-24 and 2-Ras relative to controls, but only to 77% and 62% respectively of the non-dormant germination percentage. These results demonstrate that strigol and its synthetic analogues have plant growth regulatory activity in non-parasitic species, suggesting that the control of germination by these compounds is via a pathway which is not unique to parasitic plant seeds.

The results of 3.1.2.3 demonstrate that GR-24 is active in stimulating seed germination of *S. hermonthica*. Although percentage germination is low compared to that obtained with host root exudate this may be an artifact of the germination assay procedure. While various methods, including change of solvent from acetone to DMSO, were employed in an attempt to increase germination, none resulted in levels equivalent to those obtained using either the method for gas collection from GR-24 treated samples or that used for germination of samples

before transfer to the oxygen electrode cuvette. It is particularly perplexing that utilization of these methods - either germination in borosilicate glass vials of a smaller volume or germinating on filter paper in petri dishes did not achieve levels higher than those presented. It is possible that, since these germination assay methods use less seed than either the experimental method used to measure O_2 uptake or the method used for ethylene measurement, stimulation of germination by GR-24 is increased by some form of seed/seed interaction, perhaps due to stimulation by higher ambient ethylene concentrations. It should be pointed out that low germination levels are not unusual in themselves - there are numerous studies in the literature were germination was in the region 40-60%, possibly due to the fluctuating germination response of *Striga*. Not withstanding the germination levels obtained, GR-24 greatly stimulated the production of ethylene by conditioned seed of *S. hermonthica.*

In contrast to stimulation by host root exudate, incubation with GR-24 causes an increase in ethylene production to 37 $\pm$ 7 nL $vial^{-1}$ $gram^{-1}$ by 3 h compared to zero ethylene after 3 h incubation in host root exudate, increasing to 136 $\pm$ 31 nL $vial^{-1}$ $gram^{-1}$ by 6 h, at which time radicle protrusion was first apparent. GR-24 stimulation of ethylene production by *S. hermonthica* was inhibited approximately 90% by 50μM AVG and 100% by 1mM AVG. Although actual germination counts were not made, only a very low percentage of seeds germinated in GR-24 containing 1mM AVG. NDE had no effect on GR-24-stimulated ethylene production. The effects of AVG and NDE on GR-24-stimulated ethylene production and germination are comparable with those obtained using host root exudate as stimulant. It was not known whether GR-

24/strigol-like compounds existed in the root exudates of host-plants at the time of these experiments, but it has been demonstrated recently that compounds active in the germination of *Striga* seed, very similar to strigol, exist in the root exudates of *Sorghum vulgare* (Hauck *et al.*, 1992). This discovery, in addition to the similarities between the mode of action of host root exudate and GR-24, ie. stimulation of ethylene production, and the resulting morphology of the germinated seeds, validate the use of GR-24 as a substitute for host root exudate. *Striga* seeds stimulated to germinate by host root exudate, GR-24 or ethylene often exhibit a characteristic curvature of the growing radicle during the period immediately following protrusion (Fig. 3.5D), which in some cases results in coiling of the radicle. This curvature of the radicle is not due to gravitational effects and appears to be separate from the chemotropic growth exhibited by the *Striga* seedling towards host root exudates (Williams, 1960, 1961ab, 1962). Seedlings which germinated at the outer limit of the germination zone showed increased extension growth and no root hair formation while those germinating at <4 mm from the host root showed decreased lateral extension, increased radicle diameter and many root hairs. While it is unknown how the effects of germination and haustorial-initiating stimulants interact to modulate the growth of the *Striga* radicle, there is evidence for the involvement of ethylene, which is known to cause characteristic coiling of some roots (Roberts, 1951; Curtis, 1968) in addition to inhibition of elongation.

Higher plants synthesise ethylene from the protein amino acid methionine, which is first converted to SAM then to ACC and then to ethylene as detailed in 1.2 and Fig. 1.2. As would be expected, if the effect of AVG on *S. hermonthica*

germination is due to inhibition of the enzymatic conversion of SAM to ACC, exogenously applied ACC is able to override AVG inhibition. This effect of ACC is concentration dependent, with 50mM ACC increasing germination to levels obtained in the absence of AVG, irrespective of the concentration of AVG included in the host root exudate solution (Fig. 3.10). In addition, in the absence of host root exudate, ACC is able to stimulate germination of both conditioned seed and seed imbibed for only 24 h (Figs. 3.12 and 3.21). Since exogenous ACC acts as a substitute for host root exudate in triggering germination of conditioned seed, it can be deduced that host root exudate has its stimulatory effect prior to ACC in the ethylene biosynthesis pathway (see Fig. 1.2).

The effect of ACC on germination is a result of the stimulation of ethylene production. This is demonstrated by the inhibition of germination by cobalt ions (Figs. 3.13 and 3.22) which are known to inhibit the conversion of ACC to ethylene (Lau and Yang, 1976b; Yu and Yang, 1979) and by measurement of ACC induced ethylene production itself (Table 3.11). Although the maximum percentage achieved with ACC treatment was greater after 7 days conditioning, the ability of ACC to trigger germination after 24 h imbibition rules out the possibility that conditioning is required for obligatory functional modifications to components of the biosynthesis pathway after ACC.

ACC has been used to trigger seed germination in non-parasitic species which have a requirement for endogenous ethylene to stimulate germination. ACC can replace the requirement for endogenous gibberellic acid (GA) in the germination of *A. caudatus* seeds (Kepczynski, 1986a,b). In this study ACC or ethephon treatments were able to overcome germination inhibition caused by the

inhibition of GA biosynthesis, to a greater degree than the application of exogenous GA_{4+7}. In addition, treatment of *A. caudatus* seeds with ACC, or the ethylene releasing compound, ethephon was found to reverse germination inhibition by abscisic acid (ABA) and/or osmotic restraint (polyethylene glycol). Germination of *L. sativa* seed is similarly stimulated by ACC. Ethylene can prevent or overcome the inhibitory effects of high temperatures or an osmoticum on *Lactuca sativa* germination (Sharples, 1973; Rao *et al.*, 1975; Braun and Khan, 1976; Negm and Smith, 1978; Abeles, 1986). Treatment with ACC also stimulated pre-germination ethylene production which induced germination in thermoinhibited *L. sativa* seeds (Khan and Prusinski, 1989) although ACC had no effect on seeds incubated at 25°C, at which temperature seeds germinate readily to $>90\%$. Although ACC greatly stimulated ethylene production by *L. sativa* seeds at either 25°C or higher, supra-optimal temperatures (Khan and Prusinski, 1989), and presumably had a similar effect on ethylene production by *Amaranthus caudatus*, no mention was made in these two studies of any morphological differences between ACC treated seeds and those which germinated upon imbibition in water. As detailed in 3.1.2.2 and 3.1.3. *S. hermonthica* seeds incubated in ACC, alone, or after host root exudate and AVG treatment, showed pronounced shortening of the radicle and reduced cell elongation at all ACC concentrations. Since the stimulatory effect of ACC on germination is due to a stimulation of ethylene biosynthesis, the morphological differences observed as a result of ACC treatment could be due to some aspect of this stimulation. Ethylene is known to affect the growth of roots, with high concentrations causing a reduction in length and increase in diameter. While low

concentrations of ethylene have been found to stimulate the growth of roots (Konings and Jackson, 1979) it is more common for ethylene to inhibit root elongation (Chadwick and Burg, 1967; Curtis, 1968). In addition to the inhibitory effect of applied ethylene (or ethylene releasing compounds) it has been shown that ACC causes root growth inhibition through the stimulation of ethylene production (Eliasson and Bollmark, 1988; Eliasson *et al.* 1989; Ortuno *et al.* 1991; Bertell and Eliasson, 1992). As discussed in 1.1.4.3 some growth regulators belonging to the coumarin family, which are commonly regarded as germination inhibitors, have been shown to stimulate the germination of *Striga* (Worsham *et al.,* 1962). Of the many coumarin derivatives tested only two caused any stimulation of germination - hydroxycoumarin and scopoletin. At scopoletin concentrations of 5μg/L or lower and at all hydroxycoumarin concentrations, both compounds stimulated germination with a normal morphological similar to that obtained with host root exudate, however scopoletin at concentrations in excess of 5μg/L caused inhibition of radicle elongation with almost none occurring at concentrations above 20μg/L (Worsham *et al.*, 1962). Seeds stimulated by 20μg/L scopoletin showed a morphology very similar to that obtained with ACC (Worsham *et al.*, 1962). Coumarin has also been shown to stimulate the germination of dormant seeds of *Arachis hypogea* L. (Ketring and Morgan, 1971) as could kinetin and the ethylene releasing compound chloroethylphosphonic acid. Incubation of dormant peanut seeds with either kinetin or coumarin resulted in the stimulation of ethylene production (Ketring and Morgan, 1971). Therefore the effect of scopoletin and hydroxycoumarin on the germination and morphology of *Striga* is also likely to be indirect, being

primarily due to the stimulation of ethylene production.

The effect of ethylene on seed germination has been described as the stimulation of radial expansion of the hypocotyl area of the embryo (Abeles, 1986). Abeles described the hypocotyl as a source of pressure which drives the radicle through the restraining embryo; it was argued that the variability between plants in their capacity to germinate in response to ethylene treatment could be due to the effectiveness of ethylene-mediated hypocotyl expansion in forcing the radicle through the restraining endosperm/seed coat. Although treatment of seeds of *Buchnera hispida* a hemiparasite not requiring host root exudate for stimulation of germination, with the ethylene releasing compound, ethrel did not cause a stimulation of germination above that obtained for untreated controls, it did result in a dramatic shortening of both hypocotyl and radicle (Okonkwo and Nwoke, 1974). A similar effect of ethylene on hypocotyl growth has been shown for *Lupinus albus* L., treatment with ethephon resulted in hypocotyl length being reduced by 85% and hypocotyl diameter being increased by 40% relative to untreated control seedlings after 6 days (Sanchez-Bravo *et al.,* 1992). This growth response was suggested to be due to an inhibition of water uptake rather than an inhibition of the development of new tissue material.

The concentrations of ethylene used to stimulate germination in the experiments detailed in 3.1.2.4 were not sufficient to cause noticeable changes in the morphology of *S. hermonthica* seedlings relative to that caused by host root exudate treatment. Injecting 250μL 0.1% ethylene into the vials used for germination tests resulted in an ethylene concentration of 126μL/L and a normal morphology compared to a concentration of 3.7μL/L following treatment with

10mM ACC which results in a modified morphology. This result seems to contradict the explanations advanced for ACC modified growth as detailed above. However, it is quite likely that impaired diffusion of ethylene, produced by the seed from exogenously applied ACC, as a result of the flooded growth conditions used for the germination test and the tissue barrier itself may cause ethylene to increase to inhibitory concentrations within the tissue (Jackson, 1985). Although vials used for ethylene determinations were rotated during incubation it is likely that this did not overcome the tissue barrier.

Indirect evidence for seed coat imposed dormancy in *Striga* is provided by the results of Egley (1972). Scarification of the seed coat at the radicular end of the seed promoted germination of conditioned *Striga asiatica* in the absence of cotton root exudate (Egley, 1972). A large transverse cut across the cotyledon end resulted in protrusion of the cotyledons due to radicle elongation although the radicle did not emerge due to the restraining force of the seed coat. Incubation in sulphuric acid for short periods also induced germination which was suggested to be a result of weakening of the aleurone layer over the radicle; the embryo could be easily expressed from stimulant or acid-treated seed although only through the radicle end (Egley, 1972). The radicles of sulphuric acid-treated seed were shorter than those stimulated by cotton root exudate which may be due to ethylene interactions. It is possible that acid treatment causes oxidation of endogenous ACC to ethylene which results in a change in radicle morphology similar to treatment with hypochlorite. Treatment of conditioned seed with ethylene at 10μL/L resulted in stimulation of germination to a level equivalent to that obtained with root exudate, although the radicles were slightly shorter. Egley

(1972) also found that germination of seed imbibed for only 1 day showed a delayed germination response to all treatments. Treatment with root exudate, ethylene, sulphuric acid or radicle-end puncture resulted in 9%, 13%, 31% and 73% germination respectively, 7 days after treatment. The low response to ethylene relative to that presented for *S. hermonthica* in this thesis (Fig. 3.18) may be due to the fact that Egley exposed seed to an ethylene concentration of 10μL/L, 5 times lower than that used in the experiments described in Chapter 3.1.3. It is also possible that *S. lutea* (= *asiatica*) (as used by Egley, 1972) may differ form *S. hermonthica* in its' sensitivity to ethylene.

Application of ACC to various plant organs (excepting flowers and pre-climacteric fruit) from a number of plant species results in a marked increase in ethylene production (Cameron *et al.*, 1979; Lurssen *et al.*, 1979). These observations led to the hypothesis that the enzyme which converts ACC to ethylene (ACC oxidase) is largely constitutive and the conversion of SAM to ACC is the rate-limiting step in ethylene biosynthesis (Yang and Hoffman, 1984). In agreement with the evidence for ACC oxidase being a constitutive enzyme is the finding that application of ACC to both unconditioned and conditioned seed of *S. hermonthica* resulted in a rapid increase in ethylene production (Table 3.13). Germination in *Striga* stimulated by host root exudate, GR-24 or thidiazuron is unlikely to be controlled by a stimulation of ACC oxidase since application of exogenous ACC results in a massive accumulation of ethylene (Table 3.13) which is several fold greater than that produced following treatment with host root exudate or thidiazuron (Fig. 3.23 and 3.26).

Concomitant with the ACC stimulated increase in ethylene production and

subsequent germination, is an immediate increase in O_2 uptake by both conditioned and unconditioned seed (Fig 3.31) in contrast to the lag phase displayed by conditioned seed stimulated by GR-24 (Fig. 3.29). It is likely that the lag time observed in O_2 uptake following treatment with GR-24 is due to certain changes not required by the germination process itself since both ethylene and ACC treatments result in an immediate continuous increase in respiration, but rather changes specific to GR-24 stimulation of germination. This lag phase has no effect on the timing of germination; radicle protrusion occurs at between 6 and 8 h following stimulation regardless of the stimulant used (Figs. 3.4, 3.12, 3.17, 3.18 and 3.19). Such a situation might be expected if treatment with GR-24 stimulates the *de novo* synthesis of ACC synthase, resulting in an increase in endogenous ACC which is then converted to ethylene by the constitutive enzyme, ACC oxidase. If this were the case, the rate of conversion of SAM to ACC is stimulated rapidly upon GR-24 treatment since ethylene production increases substantially in the first 3 h of incubation (Fig. 3.25). The putative increase in the concentration of free ACC after host root exudate, GR-24 or thidiazuron treatment is not sufficient to saturate ACC oxidase since the amount of ethylene produced as a result of these treatments is many fold less than that obtained with an exogenous supply of ACC (Table 3.13).

The period immediately prior to germination triggered by GR-24 is not typified by greatly increased O_2 uptake, whereas the rate of O_2 uptake increases rapidly after radicle protrusion (compare Figs. 3.17 and 3.29). It is possible that this increase in O_2 uptake is due to the increased O_2 availability resulting from puncturing the seed coat. However, the other germination stimulants used in this

study cause a more rapid increase immediately following their application, hours before radicle protrusion (Figs. 3.30, 3.31 and 3.32). Germination in most species of angiosperm has been said to be a result of cell enlargement and not necessarily division (Bewley and Black, 1983). Raghavan and Okonkwo (1982) demonstrated that most growth in the embryo of *S. gesnerioides* and *Alectra vogelii* during the early phase of germination is due to cell elongation. Assuming that a similar situation exists in *S. hermonthica*, it may be that cell elongation requires less O_2 than cell division with respect to oxygen usage.

It has been known for many years that germination of seeds of *S. asiatica* could be stimulated by kinetin and other 6-substituted aminopurines (Worsham *et al.*, 1959). The most active compound tested was 6-phenylaminopurine followed by 6-(2-Phenylethyl) aminopurine then 6-benzylaminopurine. The latter showed a germination maximum at 44μM of 70% decreasing to 12% at a concentration of 111μM. Cytokinin-type compounds are known to stimulate ethylene production in a number of plant tissues (Fuchs and Lieberman, 1968; Lau and Yang, 1973, 1974, 1976a; Yu *et al.*, 1981).

In addition to the cytokinins mentioned, the defoliant thidiazuron (*N*-phenyl-*N'*1,2,3-thidiazol-5-ylurea), which had previously been shown to have cytokinin activity in promoting the growth of cytokinin-dependent callus cultures of *Phaseolus lunatus* cv. Kingston (Mok *et al.*, 1982) was demonstrated by Suttle (1984) to stimulate ethylene evolution from etiolated mung bean hypocotyls. The stimulation of ethylene production in mung bean hypocotyls could be suppressed by inhibiting either ACC synthase (with AVG, α-aminoisobutyric acid or aminooxyacetic acid) or ACC oxidase (with $CoCl_2$) (Suttle, 1984). Although the

data suggested that ethylene production in thidiazuron treated tissues was limited by the availability of ACC, Suttle could find no corresponding stimulation of the activity of ACC synthase. However, the ability of inhibitors of ACC synthase to inhibit thidiazuron-stimulated ethylene evolution and the poor ability of *N*-malonyl conjugated ACC to act as a source of free ACC led Suttle to conclude that the increase in free ACC in mung bean hypocotyls following thidiazuron treatment is the result of *de novo* synthesis of ACC from methionine via SAM. The mode of action of cytokinins in stimulating ethylene production in wheat leaves has also been proposed to result from stimulation of ACC synthase; treatment with benzyladenine caused an increase in ethylene production and endogenous ACC level but did not affect the conversion of ACC to ethylene (McKeon *et al.*, 1988).

Cytokinin and auxin are known to act synergistically in a variety of systems to stimulate ACC synthase activity (Yang and Hoffman, 1984). Yoshii and Imaseki (1981), working with mung bean hypocotyls, showed that benzyladenine and IAA acted synergistically increased the ACC content parallel to the rate of ethylene production. Kim *et al.* (1992) reported the stimulation of ACC synthase in mung bean hypocotyls and cultured apple shoots by auxin and benzyladenine. The increase in ACC synthase activity was shown to result from the induction of ACC synthase mRNA and thus auxin and benzyladenine increase ethylene production transcriptionally (Kim *et al.* 1992).

The increase in ethylene production associated with ripening and that induced by wounding have also been shown to result from an induction of ACC synthase mRNAs (Olson *et al.* 1991). These results confirm the earlier

mechanism for stimulation of ethylene production by cytokinins proposed by Yang (1980). Yang (1980) suggested that both auxin and/or cytokinin treatments affected ethylene production by stimulation of the synthesis of ACC synthase. The ability of thidiazuron to stimulate germination in *S. hermonthica* was recently demonstrated by A.G.T. Babiker (J.J. Nour personal communication), however it was not known how thidiazuron stimulated germination. The activity of thidiazuron in stimulating germination of *Striga* seed (Fig. 3.19) is greater than that of benzylaminopurine for both *S. asiatica* (Worsham, 1959) and also for *S. hermonthica* in this study (data not shown). The results of Suttle (1984) demonstrated an elevation of ethylene production within 90 min of thidiazuron treatment whereas Lau and Yang (1973) found a lag time between application of cytokinin and the initial increase in the rate of ethylene production of around 6 h. The results of this study agree more closely with those of Lau and Yang (1973), thidiazuron treatment led to a long lag time before there was a detectable increase in ethylene production by seeds of *S. hermonthica* (Fig. 3.26). This 10 h lag period is much greater than that following application of either GR-24 (which led to an increase in ethylene production within 3 h) or host root exudate (within 6 h). It is important to acknowledge that the lag time referred to is that before detectable amounts of ethylene have been produced. Although stimulation of germination by thidiazuron can be inhibited by both AVG and, more effectively, by $CoCl_2$, ethylene is not detected until 10 to 12 h following application of thidiazuron whereas radicle protrusion was first noticed at 6 h (Figs. 3.19 and 3.26). This suggests that thidiazuron sensitizes the seed to ethylene such that a much lower concentration is active in stimulating germination. This sensitivity

mechanism may involve some aspect of the ethylene binding site since incubation in NDE results in an inhibition of ethylene production.

Thidiazuron is also effective in stimulating germination of unconditioned *Striga* seed, a result not mentioned in the studies using other cytokinins to stimulated *Striga* germination. This observation supports the theory that the conditioning process is not necessary for germination *per se* although conditioning is a prerequisite for the perception and/or transduction of the host root exudate (or analogues such as GR-24) germination signal. In addition the rate of incorporation of ^{35}S-methionine into protein during the conditioning period from 24 h to 7 days increases 2.5 times (Table 3.16). While this increase in the rate of protein synthesis is not required for germination it may, in part, result from the *de novo* synthesis of proteins necessary for the perception/transduction of the host root exudate signal.

Seeds stimulated by thidiazuron differ in their gross morphology from those treated with host root exudate, GR-24 or ethylene. Conspicuous radial swelling of the radicle occurs concomitant with the production of numerous root hairs such that the radicle resembles an haustorium. By 48 h post treatment the cotyledons have expanded and the seedling becomes separated from its testa, however no further elongation growth occurs beyond the stage shown in Figs. 3.5G and 3.7. Haustorium initiation in response to thidiazuron is typical of cytokinin active compounds (Worsham, *et al.*, 1959; Williams, 1961b) although in seed treated with other cytokinins the hypocotyl elongates within 4 or 5 days with elongation of the shoot apex (Worsham *et al.*, 1959). The morphology shown in Figs. 3.5G and 3.7 is occasionally seen after host root exudate

treatment; Williams (1962) noted that about 1% of host root exudate treated seedlings showed abnormal growth typified by the growth of root hairs - it is likely that this growth pattern is as a result of higher concentrations of haustorium inducing compounds (Riopel and Vance Baird, 1987; Lynn and Chang, 1990) in the host root exudate than is normally obtained.

AVG is much less effective an inhibitor of thidiazuron than host root exudate stimulated germination (compare Figs. 3.8 and 3.20). If, as seems likely, the mode of action of thidiazuron on the ethylene biosynthesis pathway is the stimulation of ACC synthase transcription then inhibitors of ACC synthase would be less effective following cytokinin treatment than inhibitors of ACC oxidase.

It remains possible that stimulation of ethylene biosynthesis in *Striga* by host root exudate/GR-24 results from the stimulation of specific proteolysis to increase the free L-methionine concentration or via the conversion of L-methionine to SAM and it is levels of SAM that are limiting. However, methionine is not limiting (Table 3.10) and when *Striga* seeds were incubated in SAM at 250μM, 500μM or 1mM, the highest germination percentage recorded was 19.1 $\pm$ 4.4% (Table 3.10) suggesting that SAM is not a limiting factor. This means of manipulating ethylene biosynthesis has been studied in other systems. When the conversion of SAM to ACC was inhibited by AVG in auxin-treated mung bean hypocotyls, this inhibition did not result in an accumulation of SAM. Conversely, when the conversion of SAM to ACC was stimulated by auxin treatment in mung bean (Yu and Yang, 1979) or by tobacco mosaic virus infection in tobacco (De Laat and Van Loon, 1982) there was no decrease in the level of SAM. These results together with the knowledge that SAM is an

intermediate in many other reactions, such as methylation and polyamine synthesis, have led to the conclusion that increased utilization of SAM for ACC synthesis does not affect the steady state level of SAM (Yang and Hoffman, 1984). Furthermore, if SAM levels are maintained, it is unlikely that the enzyme converting L-methionine to SAM, S-adenosyl-L-methionine synthase (SMS), becomes a rate-limiting enzyme (Yang and Hoffman, 1984). However, it has been demonstrated recently that induction of mRNA encoding SMS results from fungal elicitor treatment (Kawalleck *et al.*, 1992). There is no evidence for the role of SMS in regulating ethylene biosynthesis. However, Kawalleck *et al.* (1992) suggest that in unstressed plant tissue, the level of SMS gene expression needed for various housekeeping functions of primary metabolism may not be sufficient to allow elevated rates of ethylene biosynthesis and other methylation reactions associated with pathogen responses, thus these responses may be under transcriptional control. Nevertheless, the majority of evidence indicates that the conversion of SAM to ACC is the rate limiting step.

Analysis of polypeptide changes during germination of *S. hermonthica* with different germination stimulants showed that GR-24 and thidiazuron cause a similar pattern of changes in the polypeptide composition. This in addition to their similar affects on O_2 uptake suggests a similar mode of action compared to seed treated with ACC or ethylene. These results suggest that host root exudate/GR-24 act by increasing the amount of free ACC through the stimulation of ACC synthase in a manner similar to thidiazuron.

Based on the data presented in this thesis a hypothesis for the mechanism and control of germination in *S. hermonthica* can be proposed. The natural host

derived germination triggers for *S. hermonthica* act to increase the production of ethylene by stimulating the *de novo* synthesis of ACC synthase, through induction of ACC synthase mRNA. Other stimulating treatments such as thidiazuron (cytokinin) also stimulate the *de novo* synthesis of ACC synthase, although in these cases it may be through the induction of different mRNAs. This stimulation of ethylene biosynthesis is analogous to the stress-induced stimulation of ACC synthase, and thus of ethylene biosynthesis, caused by mechanical wounding, flooding, temperature perturbations, and by chemicals such as pollutants and fungal exudates (Yang and Hoffman, 1984). It is proposed that host derived germination triggers be considered as elicitors which act in a similar way as coronatine, a toxin produced by the plant pathogenic bacterium, *Pseudomonas syringae,* which is known to stimulate ethylene production in plant tissues, with all its varied biological effects attributable to the action of this ethylene (Ferguson and Mitchell, 1985, Kenyon, 1991).

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Role of Ethylene in the Germination of the Hemiparasite *Striga hermonthica*[1]

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ABSTRACT

Seed germination of the hemiparasitic angiosperm *Striga hermonthica* (Del.) Benth is elicited by compounds present in the root exudates of the host plant. Although a variety of compounds can substitute for the host-derived signal, the mechanism through which these act is unknown. In the present study, an inhibitor of ethylene biosynthesis, aminoethoxyvinyl glycine, was found to inhibit germination. Addition of an intermediate in ethylene biosynthesis, 1-aminocyclopropane-1-carboxylic acid, was found to override this inhibition and to act as a substitute for the host-derived signal. 2,5-Norbornadiene, an inhibitor of ethylene action, was also found to inhibit germination. Ethylene is rapidly produced by *Striga* seeds after treatment with host root exudates. These results are consistent with a model for *Striga* seed germination in which host-derived signals and other compounds act by eliciting the synthesis of ethylene and in which ethylene itself initiates the biochemical changes leading to germination.

Seed germination in many species of parasitic angiosperms is unique in so far as it is triggered by compounds released from the roots of host plants (14). Seeds of the hemiparasitic angiosperm *Striga hermonthica* (Del.) Benth require a period of imbibition (2–14 d) at temperatures about 30°C, a process known as conditioning, before they have the potential to germinate. Germination occurs, however, only in response to compounds present in exudates from host roots; conditioned seeds simply kept moist do not germinate (16, 18). The first naturally occurring germination stimulant to be characterized was the sesquiterpenoid, strigol, present in root exudates of the false host, cotton (5, 6). More recently the host plant, sorghum, has been shown to exude a dihydroquinone (2-hydroxy-5-methoxy-3-[(8′*Z*,11′*Z*)-8′,11′,14′-pentadecatriene]-*p*-hydroquinone which is a very potent stimulant of germination (4, 15). A third compound capable of stimulating *Striga gesnerioides* (Willd.) Vatke seed has been identified in exudates of cowpea roots; this compound consists of a xanthine ring, an unsaturated C_{12}-carboxylic acid, and a dipeptide of glycine and aspartic acid (10, 20). Several other compounds are reported to be active in stimulating the germination of *Striga* seed (21), albeit at higher concentrations than those at which root exudate compounds are active. Stimulants identified include kinetin, zeatin, abscisic acid, scopoletin, inositol, methionine, and ethylene. The specificity and mode of action of the germination signals remain unclear (18).

Although the requirement for host derived signals to trigger germination may be unique in parasitic plants such as *Striga*, many of the compounds which illicit this response *in vitro* also promote or enhance the germination of other angiosperms. Ethylene in particular stimulates the germination of many species (2, 7, 12, 13, 19). In this study we examine the hypothesis that ethylene is the germination stimulant for *Striga* seeds and that host derived germination signals trigger germination by eliciting the biosynthesis of ethylene.

MATERIALS AND METHODS

Plant Materials, Growth Conditions, and Chemicals

Seeds of *Striga hermonthica* (Del.) Benth were collected from Wad Medani, Sudan, in 1989. Seeds were surface-sterilized by washing in a 5% v/v solution of sodium hypochlorite (10–14% w/v available chlorine). Conditioning of seed took place on two sterile circles of Whatman No. 1 filter paper, saturated with sterile double-distilled deionized water, in 9 cm perspex Petri dishes incubated at 33°C in the dark for 7 d. Unconditioned seeds were treated as above except incubation was for 24 h only. Throughout the conditioning period the filter papers were saturated with sterile double distilled deionised water. For host root exudate collection, seeds of *Sorghum bicolor* (L.) Moench cv CSH-1 were surface-sterilized as above and germinated on moist sterile Whatman No. 1 filter paper at 33°C, after 48 h the germinated seeds were placed on nylon grids suspended over 500 cm^3 plastic beakers containing a 20% Long Ashton solution (11), so that the radicles were immersed in the solution. Plants were grown in a controlled environment (day temperature 35°C, night temperature 20°C, 16-h day length, light intensity at bench level of 103 Wm^{-2}). Four days later, three plants were transferred to 50 cm^3 of sterile double-distilled deionized water and incubated at 20 to 25°C in the dark for 24 h; this solution was used as germination stimulant. All chemicals were of analytical grade or higher. AVG[4] and ACC were purchased from Sigma while NDE was purchased from Aldrich.

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[4] Abbreviations: AVG, aminoethoxyvinyl glycine; ACC, 1-aminocyclopropane-1-carboxylic acid; NDE, 2,5-norbornadiene.

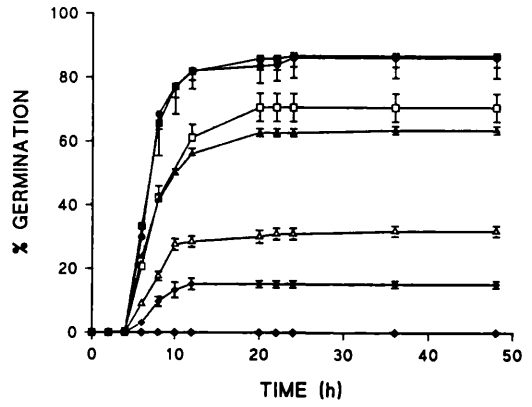

Figure 1. Effect of AVG on *Striga* germination. Seeds were incubated in host root exudate containing different concentrations of AVG: ●, O μM; ■, 1 μM; □, 5 μM; ▲, 10 μM; △, 50 μM; ♦, 100 μM; ◇, 1 mM. Error bars indicate ± SD (n = 12).

Measurement of Germination

Test solutions (200 μL) and *Striga* seeds (10/well) were added to flat-bottomed polystyrene microtiter plates (Sterilin, Middlesex, UK) at time zero and incubated in the dark at 33°C. Each sample was replicated 12 times (*i.e.* a total of 120 seeds). Incubation media included various concentrations and combinations of host root exudate, AVG and ACC. For tests involving NDE the seeds (10/vial) were placed in 1.95 cm^3 silicone rubber sealed vials (Life Science Laboratories Ltd., Luton, UK) together with 200 μL exudate. Gas samples of 10 or 100 μL of NDE stock were immediately injected, and the vials were rotated end to end at 1 revolution s^{-1} in an incubator at 33°C. NDE stock was prepared by injecting 18 μL of NDE liquid into a 8.87 cm^3 silicone rubber sealed vial; this was left for 1 h at room temperature before use. Sterile double-distilled deionized water controls were included in all experiments. For measurement of ethylene-stimulated germination, seeds (10/vial) were placed in gas-tight vials as detailed for NDE above; with 400 μL sterile double-distilled deionized water replacing host root exudate. Immediately after sealing, 100 μL of 0.1% ethylene were bubbled through the water using a gas-tight Hamilton syringe and the vials incubated as above. Addition of NDE or ethylene took place at time zero. Germination counts based on radicle protrusion were made at intervals up to 48 h after the last treatment. All experiments were repeated at least three times and found to be reproducible. Results presented are a typical set of twelve replications. Mean percentages and standard deviations were calculated from arcsine transformed data.

Gas Analysis

For each sample 30 mg of seed were surface-sterilized and conditioned as above, washed with sterile double-distilled deionized water and transferred to a 8.87-cm^3 vial containing a final test solution volume of 1.5 cm^3. Each vial was placed in the incubator for 1 min for temperature equilibration before being sealed as above and rotated end to end at 1 revolution s^{-1}. At intervals, 5 cm^3 of the head-space were withdrawn from a vial, using a gas-tight Hamilton syringe, for ethylene measurement. Values were corrected for dilution due to sampling method and new vials were used for each sampling time. Concentrations were determined by gas chromatography using a Poropak R (80–100 mesh) column and a flame ionization detector. Flow rates and temperatures were set as previously described (9).

RESULTS AND DISCUSSION

The germination of *S. hermonthica* seeds triggered by root exudates from sorghum roots was detectable after 6 h and had reached its maximum by 20 to 24 h (Fig. 1). Germination was found to be inhibited by concentrations of AVG greater than 5 μM and was completely inhibited at 1 mM (Fig. 1). AVG had little effect on the time course of germination, but altered the final percentage germination. The addition of ACC to seeds incubated for 24 h with AVG brought about a rapid increase in germination, a response to ACC being observed within 6 h (Fig. 2). The germination of ACC treated seeds reached its maximum 24 h after the addition of ACC. Concentrations of ACC above 100 μM were found to stimulate germination of Striga seeds and at 50 mM ACC gave levels of germination comparable with those obtained with root exudate alone (Fig. 3). Germination triggered by sorghum root exudates was also found to be inhibited by NDE (Fig. 4), which is known to inhibit ethylene mediated responses (17). NDE stock at 10 μL/vial reduced germination by over 50% although increasing the concentration of NDE 10-fold resulted in no further reduction. Conditioned seeds stimulated with ethylene showed a similar response to those stimulated by host root exudate (Fig. 5). Seeds which had imbibed for only 24 h also responded to ethylene treatment although there was a 12-h lag period before germination (Fig. 5).

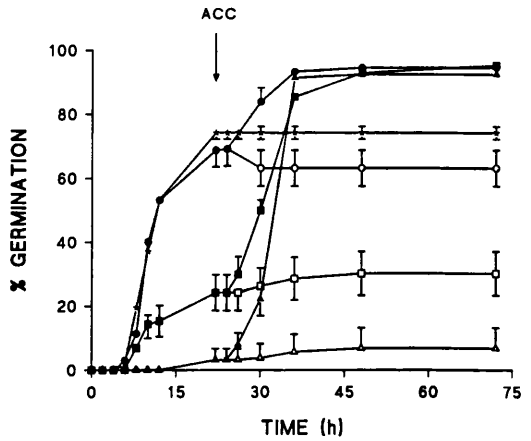

Figure 2. Overriding AVG germination inhibition by addition of ACC. Seeds were incubated for 24 h in host root exudate containing either 0.1 μM AVG (●), 50 μM AVG (■), or 1 mM AVG (▲) before addition of either 50 mM ACC (closed symbols) or 100 μM ACC (open symbols). Control treatment, ★, was of host root exudate only. Error bars indicate ± SD (n = 12).

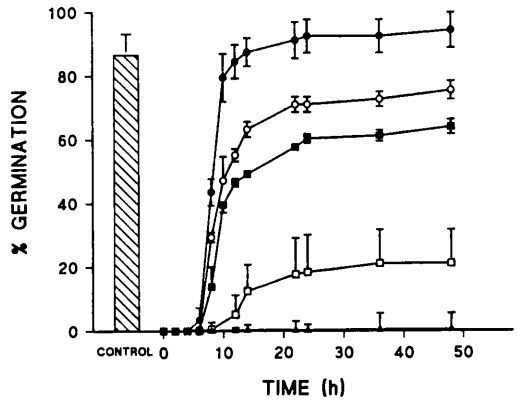

Figure 3. Effect of ACC on *Striga* germination. ACC concentrations were: ●, 50 mM; ○, 10 mM; ■, 5 mM; □, 1 mM; ▲, 100 μM. Control bar represents germination with host root exudate at 48 h. Error bars indicate ± SD (n = 12).

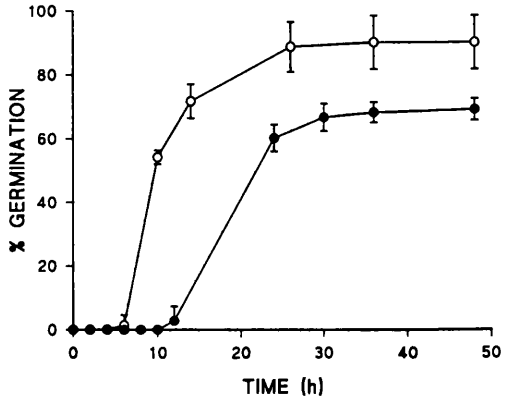

Figure 5. Time course of unconditioned, ●, and conditioned, ○, *Striga* seed germination in response to exogenous ethylene as detailed in the "Materials and Methods." Error bars indicate ± SD (n = 12).

Sterile double-distilled deionized water controls gave zero germination.

Striga seed germination triggered by sorghum root stimulants is blocked by an inhibitor of ethylene biosynthesis (AVG) which has been shown by Adams and Yang (3) to inhibit ACC synthase. This inhibition is completely overridden by an intermediate of ethylene biosynthesis (ACC) which is the product of the reaction inhibited by AVG. Moreover, ACC can not only override the AVG inhibition of germination but it can also substitute for host root stimulant in promoting the germination of dormant *Striga* seeds. Similar responses to AVG and ACC have been observed with *S. hermonthica* seed from the same region, collected in 1981 and 1987. These results suggest that the mode of action of the germination stimulants exuded by sorghum roots could be indirect, through activation of the synthesis of ethylene. Consistent with this suggestion is the observation that NDE also inhibits the germination of *Striga* seeds triggered by host root exudate. Silver ions in the form of silver thiosulfate also inhibit *Striga* seed germination (data not shown).

Further evidence for the endogenous role of ethylene is provided by the similarities between the response of seeds challenged with either host root exudate or exogenous ethylene. Unlike host root exudate, however, ethylene promotes germination of seeds not subjected to a prolonged conditioning period. This suggests that the conditioning process is not a prerequisite to ethylene perception or transduction of its biochemical effects.

Application of host root exudate induces the production of ethylene by *Striga* seeds within 6 h (Fig. 6), and production was at its maximum after 9 h incubation. As might have been anticipated, AVG substantially inhibited, while ACC greatly

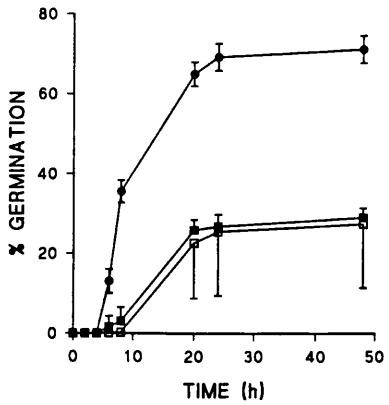

Figure 4. Effect of NDE on *Striga* germination. Seeds were incubated in host root exudate with NDE added at two concentrations as described in "Materials and Methods," ■, 10 μL/vial; □, 100 μL/vial. Control treatment, ●, was of host root exudate. Error bars indicate ± SD (n = 12).

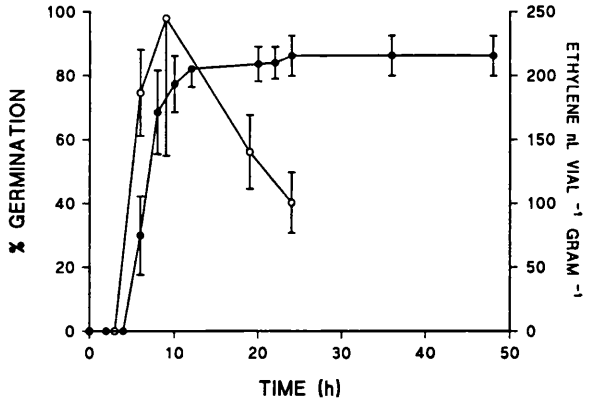

Figure 6. Time course of *Striga* seed germination, ● (n = 12), and ethylene production, ○ (n = 3), by *Striga* seeds after incubation with host root exudate. Seeds were added to host root exudate at time zero. Germination and ethylene production were measured as described in "Materials and Methods." Error bars indicate ± SD.

Table I. *Effect of AVG, ACC, and NDE on Ethylene Production*

Ethylene production was measured as described in "Materials and Methods." For ACC, readings were first made at 6 h then the vials were requilibrated with air at 33°C, returned to the incubator and sampled 24 h later. The control treatment was of host root exudate. Seeds were added to the test solution at time zero. Values are means of three replicates ± SD.

Treatment	Ethylene Production		
	6 h	24 h	30 h
	nL vial^{-1} *g*$^{-1}$		
Control	186 ± 34	101 ± 24	
10 mM ACC	1082 ± 56		6256 ± 15
100 μM ACC	Not detected		68 ± 18
1 mM AVG	Not detected		
50 μM AVG	Not detected	15 ± 9	
NDE	274 ± 18		
Distilled H_2O	Not detected	Not detected	

stimulated, ethylene production (Table I). NDE was found to slightly stimulate ethylene production by *Striga* seeds.

The production of ethylene by *Striga* seeds challenged with host root exudate is consistent with the hypothesis that the host derived trigger stimulates ethylene biosynthesis which then triggers *Striga* seed germination. Observations that the addition of ACC stimulates the germination of seeds in the absence of root exudate and that AVG inhibits germination suggests that the chemical stimulus from the host has its effect prior to ACC in the ethylene biosynthetic pathway.

We have also observed that the *Striga* seeds stimulated to germinate by ACC have very short radicles and seem thicker than those which germinate in response to host root exudate. This response to ACC may be due to inhibition of root elongation by the high concentrations of ethylene generated (1, 8).

Some of the chemicals present in sorghum root exudates which trigger germination of *Striga* seeds have been shown to have allelopathic properties, inhibiting the germination of other seeds (15). It is tempting to speculate that their effect on *Striga* seed germination represents a modification of the plant wound syndrome in which wounding, chemical or mechanical is accompanied by a burst of ethylene synthesis (2).

The results presented here are consistent with a model for the control of *Striga* seed germination in which a variety of chemical signals derived from host root exudates act through a common mechanism, namely the elicitation of ethylene biosynthesis and it is ethylene itself which initiates the biochemical cascade which results in germination.

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INVITED REVIEW

Germination of the seeds of parasitic angiosperms

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Abstract

Totalling more than 3000 species, parasitic angiosperms are a most diverse group of organisms. Classified as either shoot or root parasites according to site of attachment, plant parasites are further classified, according to the degree of parasitism, into one of two divisions: holoparasitic or hemiparasitic. Within these divisions, angiosperm parasites are either obligate or facultative.

Obligate parasites cannot survive to maturity without attaching to a host plant, thus the need to germinate in close proximity to a host first characterizes the specialized germination and seed dispersal strategies of these plants. However, seeds of all species of the root-holoparasitic Orobanchaceae and four genera within the Scrophulariaceae have evolved very specialized germination strategies. Once the seeds have undergone an after-ripening period, they require a prolonged imbibition period at temperatures of approx. 30°C before they acquire the potential to germinate. Minute seeds with very little reserves, they must germinate within millimetres of the host root for successful host attachment and continued survival. To this end, germination only occurs in response to specific chemical germination triggers released from the host root. In *Striga hermonthica* (Del.) Benth., the perception of the host-derived trigger results in stimulation of endogenous ethylene production and it is this ethylene that initiates germination. It is proposed that the chemical triggers released from the host be considered as 'elicitors' which function primarily as allelochemicals and that the parasite seeds have become adapted in such a way as to have an absolute requirement for external germination triggers.

Keywords: *Striga*, *Orobanche*, ethylene, allelochemicals

Introduction

Forming 1% of the total number of all flowering plants, parasitic angiosperms are a most diverse group of organisms. With individual species totalling more than 3000, distributed within 17 plant families, parasitic plants are found throughout the Old and New Worlds, (Kuijt, 1969).

Parasitic plants rely on their host for some, if not all, of their nutrients. Attaching to either roots or shoots, they capture water and solutes from the host via the haustorium, a novel organ common to all parasitic angiosperms. While the haustorium has a similar physiological function in different species, it is a highly variable organ morphologically and anatomically.

Parasitic angiosperms are frequently divided into one of two groups, holoparasitic or hemiparasitic, although only at the extremes of each are they truly distinct. Holoparasites depend almost entirely on their host for their nutritional requirements. Lacking chlorophyll and thus unable to photosynthesize, they are all obligate parasites. Hemiparasites can be classified, according to their degree of host dependence, as either facultative or obligate parasites. Facultative hemiparasites are capable of surviving and setting seed in the absence of a host although most are parasitic in their natural habitats, e.g. *Rhinanthus*, *Bellardia*, *Euphrasia*, *Odontites*. Obligate hemiparasites are partly dependent on their hosts for their nutritional requirements while being completely dependent on their host for survival. All hemiparasites are chlorophyllous and were traditionally thought only to rely on their host for water and minerals. However, there is frequently substantial movement of carbon from host to parasite (Press *et al.*, 1987).

Parasitic angiosperms are further classified by their site of attachment to the host. Shoot parasites

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include the holoparasitic dodders, *Cuscuta* spp., belonging to the family Convolvulaceae, the evolutionarily distinct scrub dodders, *Cassytha* spp., belonging to the family Lauraceae, and hemiparasites such as mistletoes of the families Loranthaceae and Viscacea, which include the well-known Christmas mistletoe, *Viscum album* L. and its North American counterpart *Phroradendron serotinum* L. Root parasites range from the holoparasitic Orobanchaceae (broomrapes) and Rafflesiaceae to the hemiparasitic mistletoe *Nuytsia* spp. found in Australia, and the largest family of root hemiparasites, the Scrophulariaceae or figworts.

Although the divisions described above provide a means of classification according to mode of parasitism it is of more relevance, for the purpose of this review, to classify individual parasites by means of their seed dispersal and germination strategies. Using the classification of Kuijt (1969), we are able to segregate parasites into one of three dissemination/germination strategies although there is some overlap between groupings.

Large seeds

The least specialized strategy involves the production of a few large seeds, such as those of *Rhinanthus*, *Cuscuta*, *Cassytha* and *Melampyrum*. These seeds contain sufficient reserves to allow extensive radicle growth while seeking a host. Dispersal mechanisms are generally haphazard or, as in *Lathraea*, the seeds are expelled violently from the capsule when it dehisces (Beck von Mannagetta, 1930). Germination of *Cuscuta* seeds in the laboratory has frequently entailed treatment with sulfuric acid (Gaertner, 1950) or grinding with glass powder (Walzel, 1952) to overcome seed-coat-imposed dormancy mechanisms. However, these requirements are by no means universal in the *Cuscuta* genus. Some desert species germinate precociously (Kuijt, 1969) while, in *Cuscuta campestris* Yunker, germination of a seed generation is spread over several years (Dawson, 1965). These mechanisms are not unique to parasitic angiosperms and are therefore considered unspecialized compared with non-parasitic angiosperm seeds.

Animal dispersal

The second strategy relies on animal dispersal to ensure that the parasite contacts a host. Many seeds in this group are surrounded by fleshy tissue often brightly coloured making them attractive to fruit-eating animals, especially birds. The seed is frequently protected by a tough seed coat which allows passage through the intestinal tract of animals without damage. This method is common in the epiphytic mistletoes, *Phacelaria*, the large seeds of which allow the production of a substantial haustorium for attaching and penetrating the woody host tissue. As a result of the necessary size of these seeds, there is usually only one per flower although the large fleshy fruits of *Prospanche americana* in the family Hydnoraceae may contain as many as 35 000 minute seeds each (Cocucci, 1965). These large fruits are known to be eaten by baboons, jackals, foxes, porcupines, and in South America by armadillos as well as the local people. Ants are also known to be involved in the dispersal of parasitic angiosperm seeds. Ants have been observed carrying the seeds of *Mystropetalon* to their nest, and fruits have even been retrieved from the nest, although without the elaiosome; seeds of *Tozzia* and *Pedicularis* are also known to be dispersed by ants (Kuijt, 1969). In *Rafflesia*, it has been suggested that seeds are dispersed by both elephants and ants (Kuijt, 1969)!

At the extremes of animal dispersal mechanisms are two species of bird, belonging to the order Passeriformes, which are thought to rely entirely on mistletoe berries for food. The stomach of the 'mistletoe bird', *Tanagra musica scateri*, from Puerto Rico has become adapted to its solitary diet as has that of the flower-pecker *Dicaeum celebicum*. The mistletoe seed passes through the digestive tract of these birds undamaged after which it may be deposited on a branch where germination and attachment can begin. Again, the germination strategies (as opposed to the dissemination strategies) of these parasites are unspecialized.

Stimulation by host

The third strategy is unique to parasitic plants and involves a highly specialized mechanism by which the parasite seed is stimulated to germinate by chemical triggers derived from the host plant. All of the Orobanchaceae, four genera of the Scrophulariaceae (*Striga* (except S. *euphrasioides*), *Alectra*, *Lathraea* and *Tozzia*), most Rafflesiaceae, Balanophoraceae and Hydnoraceae require the presence of host-derived chemicals to stimulate germination. The success of this mechanism hinges on the production of vast numbers of minute seeds (approx. 350 × 230 μm in *Striga*) which are dispersed by wind and water. Many of the species within this grouping are serious agricultural pests, particularly in the Developing Countries, where they attack and devastate cereal and legume crops. Such parasitism results in reduction of shoot growth, severe wilting and chlorosis, which contribute to yield reductions of up to 100% in *Sorghum* infected with *Striga hermonthica* (Del.) Benth. (Doggett, 1965).

The vast majority of parasitic plant research has been conducted on agriculturally important genera. Most commonly these are obligate parasites. *Alectra vogelii* Benth. is a serious parasite of legume crops in Africa, while other *Alectra* species can attack tobacco,

sunflower and other crops (Wild, 1954). One genus of the Orobanchaceae, *Aeginetia*, is known to attack sugarcane, sorghum and millet. Facultative parasites of the Scrophulariaceae, such as species of *Rhinanthus* and *Odontites*, have caused limited growth losses in some areas in the past, but their importance has never reached that of *Striga* and *Orobanche*.

Although parasites of economic importance include both holo- and hemiparasites, almost all are root parasites. For these reasons, this review will concentrate on the root parasites belonging to the Scrophulariaceae and Orobanchaceae, with reference to others when of particular interest.

After-ripening and dormancy

In common with the majority of non-parasitic angiosperm weeds, most parasitic weed seeds have some form of dormancy mechanism. Where specialized (host-induced) germination strategies have evolved, the seeds must first undergo a period of after-ripening. Defined as any internal changes that take place after the seeds are shed and that lead to germination or increased germination, after-ripening is a period of dormancy during which germination will not occur (or occurs only to a limited extent) even under optimal conditions (Bewley and Black, 1982). This period, which generally lasts for up to 6 months, is a common feature of *Striga* and *Orobanche*, the most economically important genera. Little is known concerning the nature of the after-ripening period or the changes which lead to the release from this dormancy mechanism.

Once after-ripened, seeds of *Striga* and *Orobanche* require a period of imbibition at temperatures ranging from 20 to 35 C for 2–14 d before they acquire the potential to germinate in response to host-derived chemical triggers. This imbibition period was first characterized by Brown and Edwards (1944), who coined the term 'pretreatment'. The terms preconditioning, pre-exposure and conditioning have also been used for the same process. The seeds should now be considered as quiescent, since germination will occur under favourable conditions, i.e. the presence of an external chemical stimulus. Although the optimum temperature for, and length of, conditioning vary between species and even between different samples of the same species, the conditioning process is an absolute requirement for many taxa within the Scrophulariaceae and Orobanchaceae (*Striga asiatica* L. Kuntze (= *lutea*): Brown and Edwards, 1944; *S. euphrasioides* Benth.: Rangaswamy and Rangan, 1966; *S. hermonthica*: Vallance, 1950; *S. densiflora* Benth.: Reid and Parker, 1979; *Sopubia delphinifolia* G. Don.: Shivanna and Rangaswamy, 1976; *Orobanche ramosa* L.: Saghir, 1986; *O. minor* Smith and *O. crenata* Forsk.: Edwards, 1972).

Much research has been carried out on the conditioning process, most workers concentrating on length of the conditioning period and the optimum temperature for conditioning (Brown and Edwards, 1946; Botha, 1950; Vallance, 1950, 1951; Reid and Parker, 1979). While it is not known what changes occur during the conditioning process, there is some evidence, in *Striga*, for the role of endogenous germination inhibitors (Williams, 1959; Kust, 1966). The results of Hsiao *et al.* (1979) demonstrated the interactions among seeds during conditioning: they found that increasing the number of seeds per dish or a smaller volume of water during conditioning increased subsequent germination on stimulation by strigol (a stimulant collected from cotton, see later), results which seem to contradict the inhibitor theory. However, these correlations were only apparent at threshold strigol concentrations.

Seeds of *Aeginetia indica* L. are also dormant following seed shedding. Dormancy in these seeds can be broken by treatment with sodium hypochlorite, exposure to low temperatures (3–5°C) for several days (stratification), or a short exposure to higher temperatures (15 min at 50°C) (French and Sherman, 1976). Whether this dormancy mechanism can be equated with the after-ripening period required by species of *Striga* and *Orobanche* or with the quiescent period following after-ripening is unclear. Kusano (1908) found that *Aeginetia* required the presence of a host, or roots of a certain non-host before germination would occur whereas French and Sherman (1976) claim that host/non-host presence is unnecessary. However, French and Sherman treated seed in their experiments with sodium hypochlorite as standard, a procedure which itself could result in germination (see section on non-growth regulators, below), or may potentiate the effects of other treatments. After initiation of germination in *Aeginetia*, the presence of a root of a host (e.g. sugarcane, *Saccharum officinarum* L.) or non-host (e.g. pea, *Pisum sativum* L.) stimulates formation of fine tendril-like protuberances which function in host attachment (Kusano, 1908; French and Sherman, 1976). Stratification has also proved effective in other species, such as *Aureolaria* (Musselman, 1969), *Castilleja* (Malcolm, 1966), *Euphrasia* (Yeo, 1961), *Odontites* (Govier and Harper, 1965), *Rhinanthus* (Vallance, 1952) and *Melampyrum* (Curtis and Cantlon, 1963). In *Melampyrum lineare* Desr., an annual root hemiparasite, germination requires two essential steps: firstly, activation at approx. 20°C followed by exposure to low temperatures (4°C) for 6–8 weeks (Curtis and Cantlon, 1963). Activation at 20°C results in up to 40% of a seed batch germinating after subsequent cold treatment, while treatment with gibberellic acid induces up to 100% germination. Although the ecological importance of this mechanism, whereby the germination of the seed crop of an annual parasite is spread over a number of years (germination occur-

ring after each winter), thus increasing the likelihood of the seed encountering a suitable host, is understood, the reasons for the embryo's additional requirements for endosperm cell wall hydrolysis (i.e. the requirement for exogenous GA to maximize germination) and its cold dependence remain unknown (Curtis and Cantlon, 1968).

If the period of conditioning is extended greatly beyond the optimal time, the seeds of *Striga* have been reported to enter a period of 'wet dormancy' (Vallance, 1950), which can be alleviated if the seeds are dehydrated and conditioned once more. Wet dormancy is an example of secondary dormancy as opposed to the primary or innate dormancy mechanisms mentioned above. Secondary dormancy is often induced, as in this case, when the seeds are given all but one of the conditions necessary for germination (Mayer and Poljakoff-Mayber, 1989). The onset of secondary dormancy has been reinvestigated by Pieterse *et al.*, (1984), who also found that germination decreases after a prolonged period of conditioning, although the period required was much greater than that reported by Vallance (10–14 weeks compared with 3–4 weeks). Also in contrast to Vallance's results, Pieterse *et al.* found that drying and then reconditioning resulted in low germination percentages in only two out of eight samples. Hsiao *et al.* (1987) found no evidence of wet dormancy after conditioning *Striga asiatica* for 56 days. This contradiction could be due to the age of the seed populations used. Vallance (1950) noted a greater reduction in germination in 6-year-old seed compared with 1-year-old; Hsiao *et al.* used 1-year-old seed. This wet dormancy could act as a safety mechanism due to the fact that seeds which have imbibed, and are undergoing conditioning respire at 30–40 μmol O_2 h^{-1} g^{-1} dry weight compared with an average of 17 μmol O_2 h^{-1} g^{-1} dry weight by seed during the first hour of imbibition (D.C. Logan and G.R. Stewart, unpublished) and thus in the absence of a germination trigger the seed would soon run out of respiratory substrate and die.

Host-derived germination stimulants

As mentioned in the Introduction, the seeds of species in almost all genera of root-parasitic plants require stimulation of germination by chemicals exuded from the host plants' roots. This unique phenomenon was first demonstrated by Vaucher (1823) for *Orobanche* and *inter alia* by Garman (1903) with *Orobanche minor*, by Kusano (1908) with *Aeginetia indica* (see above) and by Heinricher (1917) with *Tozzia* and *Lathraea*. Pearson (1911) noted that germination of *Striga* only occurs when the seed lies very close to—probably in actual contact with—a root on which the rooibloem (*Striga*) will grow. However, Saunders (1933), working on *Striga asiatica*, was the first to demonstrate that actual contact between parasite and host root was not required, by stimulating seeds to germinate with a water-soluble exudate from maize roots. This conclusion was also reached by Brown and Edwards (1944) using a different experimental design involving *Striga asiatica* and exudate from intact seedlings of *Sorghum vulgare*.

Many different compounds are released by host and non-host plants, which are active in stimulating the germination of root-parasitic angiosperm seed, and the ability of each compound in the mixture to stimulate germination can vary with different parasite species (Sunderland, 1960; Cook *et al.*, 1972; Visser and Botha, 1974; Visser, 1975; Hauck *et al.*, 1992). Many workers have attempted to identify the germination stimulants exuded from host plants' roots, but have had considerable difficulty for two main reasons. Firstly, host-derived germination triggers are active at very low concentrations (down to fmol m^{-3} of soil solution). Secondly, these compounds are extremely labile, being very sensitive to pH changes and oxidation.

The first naturally occurring germination stimulant was reported from root exudates of cotton by Cook *et al.*, (1966). Six years later the stimulant, identified as a sesquiterpene, was given the trivial name strigol (Cook *et al.*, 1972) (Fig. 1A), but it was a further 2 years before total synthesis of the molecule was accomplished (Heather *et al.*, 1974, 1976) and yet a further 11 years before the absolute structure was established (Brooks *et al.*, 1985). The breakthrough by Cook and his colleagues offered the possibility of using chemical induction of germination as a means of clearing fields of *Striga* infestation.

Strigol is active at concentrations as low as 10^{-9} mol m^{-3} in soil solution and it was not long after its identification that structure-activity studies were conducted, which led to the synthesis of a number of analogues (termed GR compounds after G. Rosebury) and precursors (Johnson *et al.*, 1976, 1981). Most of the compounds tested were more active in stimulating germination of *Orobanche* than of *Striga* seeds. Many synthetic lactones that were active in stimulating germination of *Orobanche ramosa* had no stimulatory effect on *Striga hermonthica*; there was also a disparity in the effects of certain lactones on germination of *S. hermonthica* and *S. asiatica* (Johnson *et al.*, 1976). One of the most active analogues to date is GR-24 (Fig. 1B). Recently, Zwanenburg and co-workers have revived the structure-activity approach (Zwanenburg *et al.*, 1986; Mhehe, 1987). Simplification of the molecule to the ring framework and the use of different substituted groups, plus knowledge of the molecular shape of strigol and bioisosterism suggest that the linkage between the two lactones is vital for biological activity.

In addition to the effects on the germination of root parasitic plant seeds, strigol and several synthetic

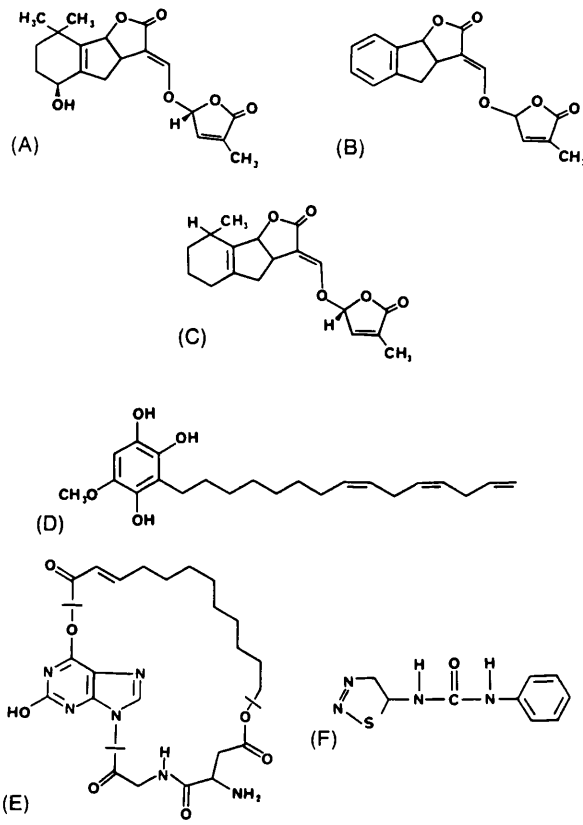

Figure 1. Germination stimulants: (A) strigol, (B) GR-24, (C) sorgolactone, (D) sorgoleone-358, (E) cowpea stimulant C-3 and (F) thidiazuron.

multi-ring analogues have been shown to stimulate the germination of dormant seed of both light-sensitive and insensitive *Lactuca sativa*, and of *Avena fatua* L., to levels significantly greater than those obtained under the same conditions with water substituting for the test solution (Bradow *et al.*, 1988, 1990). In *L. sativa*, the germination percentage obtained with GR-24 and the 2- and 3-ring strigol analogues (2-Ras and 3-Ras) was not significantly different from that obtained under conditions which overcame the endogenous dormancy mechanism. Germination of *Amaranthus retroflexus* L. was approximately doubled by GR-24 and 2-Ras relative to the percentage which germinated in distilled water under the same experimental conditions, but only to 77 and 62%, respectively, of the non-dormant germination percentage (Bradow *et al.*, 1988). The stimulatory effect of strigol on *Avena fatua* equalled or exceeded that caused by equimolar GA_3 (Bradow *et al.*, 1990). These results demonstrate that strigol and its synthetic analogues have plant growth regulatory activity in non-parasitic dicotyledons and monocotyledons, suggesting that the control of germination by these compounds is via a pathway which is not unique to parasitic plant seeds (see section on substitute germination triggers, below).

In addition to strigol and its analogues, many other sesquiterpene lactones are known to stimulate germination of *Striga asiatica*. Comparison of the stimulatory activity of ten germacranolides indicated that it may be the medium-ring skeleton and the lactone ring of these molecules which are important to this activity (Fischer *et al.*, 1990). A second naturally occurring germination trigger has been identified from the root exudates of *Sorghum vulgare*. Hydrophobic droplets on *Sorghum* root hairs were first realized to be *Striga* germination stimulants by Netzly & Butler (1986). These droplets contain a number of *p*-hydroquinones present in either oxidized or reduced form (dihydroquinone) (Chang *et al.*, 1986; Netzly *et al.*, 1988). After extraction with dichloromethane and separation by HPLC, two major components were recovered. The biological activity was related to the concentration of only one of these two compounds, which was given the name sorgoleone-358 (2-hydroxy-5-methoxy-3-[(8′Z,11′Z)-8′,11′,14′-pentadecatriene]-*p*-dihydroquinone; see Fig. 1D). This compound is very unstable and is rapidly oxidized to the quinone form although reduction back to the dihydroquinone has been shown using tin amalgam or zinc dust (Fate *et al.*, 1990). Sorgoleone-358 is active at higher concentrations than strigol (10^{-4} mol m^{-3} compared with 10^{-9} mol m^{-3}) while being much more labile. Recently, germination stimulants have been isolated from the host *Sorghum bicolor*. This complex mixture of stimulants includes two which are more active in stimulating the germination of both *Striga asiatica* and *S. hermonthica*, while another compound is more active in stimulating the germination of *S. gesnerioides* and *Alectra vogelii*. Preliminary identification of the most active component stimulating germination of *S. hermonthica* and *S. asiatica*, given the name sorgolactone, suggests a structure similar to that of strigol (Fig. 1C) (Hauck *et al.*, 1992). A fourth compound, identified from the root exudates of cowpea, *Vigna unguiculata* Walp., has been implicated in the germination of *Striga gesnerioides* (Willd.) Vatke and *Alectra vogelii* (Herb *et al.*, 1987; Visser *et al.*, 1987): this cyclic molecule consists of three parts, a xanthine, an unsaturated C_{12}-carboxylic acid and a dipeptide of aspartic acid and glycine (Fig. 1E).

The germination trigger sorgoleone-358, identified by Chang *et al.*, (1986), is also known to function in the characterization of host distance and is suggested to be responsible for the chemotropic growth response of the parasite radicle (Fate *et al.*, 1990). When *Striga* seeds were placed at distances parallel to the root of a 4-day-old sorghum seedling in an agar plate, maximum germination occurred within a radius of 5 mm, which is equal to the maximum length of the *Striga* radicle. Imaging of the hydroquinone with methylene

blue showed a clear reduced zone detectable after several days, equilibrium being reached at 5 mm after 5 days. Although establishment of a steady-state gradient can provide information concerning host presence and spatial distance, it is unknown to what extent this is maintained in the soil (Fate *et al.*, 1990). Williams (1961a,b, 1962) demonstrated the influence of host-derived chemical signals on the morphology of the germinating parasite seed and showed that not only germination but also morphology varied with proximity to the host root. In the zone nearest the host root ($\leqslant$4 mm), the rate of extension growth was very low, the parasite radicle undergoing considerable lateral expansion together with the production of root hairs. At this distance, growth of the *Striga* radicle was positively chemotropic although not all seedlings grew towards the host root. As distance from the host root increased, extension growth of the parasite radicle increased with reduced lateral expansion and root hair production. Increased production of root hairs and the redirection of cellular extension at the root meristem from a longitudinal to a radial dimension are now known to be typical features in the change from a developmental mode to a parasitic mode of growth in root-parasites. The chemical triggers involved in this process and particular events leading to haustorial initiation, parasite attachment and subsequent parasitic growth are outside the remit of this review and readers are instead directed towards recent reviews on the subject (Lynn and Chang, 1990; Press *et al.*, 1990; Stewart & Press, 1990).

Substitute germination triggers

Apart from strigol, its GR analogues and host-derived stimulants, many other chemicals are known to induce germination of root-parasitic angiosperms. Most of these compounds are plant growth regulators, having similar effects on non-parasitic angiosperm seeds.

Ethylene

The effects of ethylene on the germination of angiosperm seeds have been well documented (Toole *et al.*, 1964; Abeles & Lonski, 1969; Ketring & Morgan, 1969; Taylorson, 1979). Egley and Dale (1970) were the first to discover that ethylene could substitute for the host-derived chemical stimulus in the germination of conditioned seeds of *Striga asiatica*. Ethylene has now been shown to cause germination of *Striga hermonthica* (Bebawi and Eplee, 1986) and *Orobanche ramosa* L. (Chun *et al.*, 1979), while *Striga gesnerioides* displays a limited response (A. Antwi, D.C. Logan and G.R. Stewart, unpublished). Ethylene has been used on a substantial scale to trigger 'suicidal' germination (germination in the absence of a host) as part of a *Striga* eradication program in the USA. Injecting ethylene into infected soil can result in germination of conditioned *Striga asiatica* of up to 90% at depths up to 30 cm and at horizontal distances up to 70 cm from the point of injection (Witt and Weber, 1975).

While it was known that the ethylene precursor L-methionine slightly (20%) stimulated *Striga* germination (Worsham, 1987), it was not known what role ethylene played in the natural germination of these parasites. Recent research using *Striga hermonthica* (Logan and Stewart, 1991) has demonstrated that stimulation of germination by host-root exudates causes the production of endogenous ethylene and it is this ethylene that initiates the biochemical cascade leading to germination. In plant tissues, ethylene is synthesized from L-methionine via a pathway controlled by the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (EC 4.4.1.14) which catalyses the rate-limiting conversion of *S*-adenosyl-L-methionine (SAM) to ACC (Fig. 2). (Adams and Yang, 1979). ACC also causes germination of *Striga* in the absence of host-root exudate and this germination stimulation can be inhibited by $CoCl_2$, an inhibitor of ACC oxidase (D.C. Logan and G.R. Stewart, unpublished). The radicles of seedlings triggered to germinate with ACC show much reduced cellular elongation (Logan and Stewart, 1991), a symptom typical of high ethylene concentrations (Elliason *et al.*, 1989). Inhibition of ethylene biosynthesis or action inhibits *Striga* germination (Logan and Stewart, 1991). In addition to host-root exudates, GR-24 stimulates ethylene production in *S. hermonthica* and the germination response has been shown to result from the action of this ethylene (D.C. Logan and G.R. Stewart, unpublished; Jackson and Parker, 1991). Jackson and Parker found dissimilar effects of GR-24 on ethylene production and action when comparing the germination of *S. hermonthica* with that of S. *forbesii*. Although ethylene is produced by *S. forbesii*, inhibition of ethylene action by 2,5-norbornadiene did not lower the subsequent germination percentage. In addition, incubating *S. forbesii* seeds in 1 μl l^{-1} ethylene failed to stimulate germination. The two samples of *S. hermonthica* used by Jackson and Parker germinated to only 4.8 and 20.6%, respectively, in response to ethylene although 2,5-norbornadiene reduced germination in GR-24 by 58 and 92%, respectively. These results suggest that the link between stimulation of ethylene production and germination is more complex than a simple concentration-dependent response. As mentioned above, different species and even samples of the same species vary in their response to host root exudates: it is possible that this variation is due to differences in the seeds' ability to synthesize ethylene in response to stimulant. In contrast, the failure of *S. forbesii* to respond to ethylene may be due to a lower sensitivity to ethylene than possessed by *S. hermonthica*.

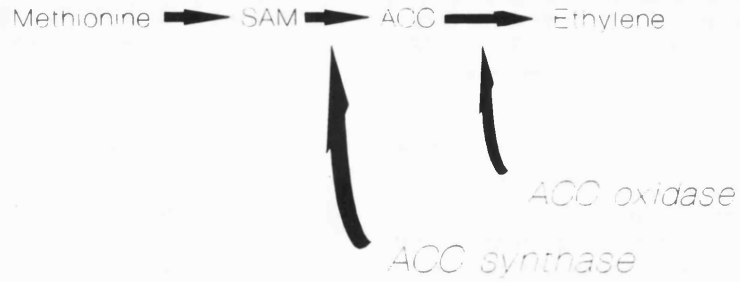

Figure 2. Ethylene biosynthesis pathway; SAM, *S*-adenosyl-L-methionine; ACC, 1-aminocyclopropane-1-carboxylic acid

Striga seeds stimulated to germinate by host-root exudate, GR-24 or ethylene often exhibit a characteristic curvature of the growing radicle during the period immediately following protrusion, which in some cases results in coiling of the radicle. This curvature of the radicle is not due to gravitational effects and appears to be separate from the chemotropic growth exhibited by the *Striga* seedling towards host-root exudates (Williams, 1960, 1961a,b, 1962). Seedlings which germinated at the outer limit of the germination zone showed increased extension growth and no root-hair formation while those germinating at <4 mm from the host root showed decreased lateral extension, increased radicle diameter and many root hairs. While it is unknown how the effects of germination and haustorial-initiating stimulants interact to modulate the growth of the *Striga* radicle, there is evidence for the involvement of ethylene, which is known to cause characteristic coiling of some roots (Roberts, 1951; Curtis, 1968) in addition to inhibition of elongation.

Cytokinins, auxins and gibberellic acid

Cytokinins have also been shown to cause germination of *Striga* seeds (Worsham *et al.*, 1959; Igbinnosa and Okonkwo, 1992). Using kinetin, benzyladenine and other 6-substituted aminopurines, Worsham *et al.* (1959) compared the efficacy of these compounds with that of a natural stimulant solution obtained from maize seedlings. At all but threshold concentrations, seeds stimulated to germinate by 6-substituted aminopurines were morphologically different from those that germinated naturally. Hypocotyls were much reduced, consisting of a bulbous mass of small unelongated cells with numerous root-hair-like protuberances, similar to the morphological changes caused by haustorial initiation; induction of haustoria by benzyladenine has also been demonstrated for *Cuscuta* spp. (Ramasubramanian *et al.*, 1988). Kinetin and zeatin stimulated germination of conditioned and non-conditioned *Striga gesnerioides* seeds, while benzylaminopurine was much less effective; all cytokinin treatments resulted in the radicles of germinated seeds being short and swollen (Igbinnosa and Okonkwo, 1992). A.G.T. Babiker (J.J. Nour, personal communication) recently found that thidiazuron (Fig. 1F), an adenine-type cytokinin, causes similar germination and seems to initiate formation of the haustorium while reducing cell expansion and elongation. Treating with kinetin *Striga* seedlings that have been stimulated with host-root exudate causes a similar morphological condition with a greater effect at lower concentrations (Williams, 1961b). Thidiazuron and benzyladenine are known to cause ethylene production in some plant tissues (Suttle, 1984; Yip and Yang, 1986), and inhibition of ethylene biosynthesis or action, concomitant with application of thidiazuron to *Striga hermonthica* seeds, results in inhibition of germination (D. C. Logan and G. R. Stewart, unpublished). The role of cytokinins in germination stimulated by host-root exudate is unknown.

Treatment of *Striga* with IAA or GA_3 failed to induce germination (Egley, 1972; D. C. Logan and G. R. Stewart, unpublished). Gibberellic acid causes germination in several species of *Orobanche* spp. in the absence of host-derived stimulus (Chun *et al.*, 1979; Pieterse, 1979). Incubation with IAA both during conditioning and at the same time as treatment with host-derived stimulant increased germination of *Orobanche aegyptiaca* Pers. (Kumar and Rangaswamy, 1977) although stimulation of germination by IAA in the absence of host-root exudate does not occur.

Coumarin, generally thought of as a germination inhibitor, has also been found to stimulate germination of *Striga hermonthica* seed (Worsham *et al.*, 1962). Of the many coumarin derivatives tested, only two caused any stimulation of germination: hydroxycoumarin and scopoletin. At scopoletin concentrations of $\leqslant 5$ mg l^{-1} and at all hydroxycoumarin concentrations, both compounds stimulated germination and the morphology was similar to that obtained with host-root exudate. Scopoletin at >5 mg l^{-1} inhibited radicle elongation and no elongation occurred with scopoletin at >20 mg l^{-1} (Worsham *et al.*, 1962). Seeds stimulated by scopoletin at 20 mg l^{-1} displayed a morphology similar to that obtained with ACC. Coumarin could also stimulate

the germination of dormant seeds of *Arachis hypogaea* L. (Ketring and Morgan, 1971) as could treatment with kinetin and the ethylene-releasing compound 2-chloroethylphosphonic acid. Incubation of dormant groundnut seeds with kinetin or coumarin resulted in the stimulation of ethylene production (Ketring and Morgan, 1971). Therefore, the effect of coumarin on the germination and morphology of *Striga* is likely to be indirect, being due to the stimulation of ethylene production.

Brassinosteroids have been shown to act synergistically with strigol, kinetin and ethephon in stimulating germination of *Striga asiatica*, with no germination as a result of brassinolide treatment alone (Takeuchi *et al.*, 1991). In addition, conditioning in brassinolide, as opposed to distilled water, resulted in a higher final germination percentage following subsequent stimulation with sorghum root exudate (Takeuchi, 1991).

Non-growth regulators

Other compounds which are not found in plant-root exudates have been shown to cause germination of *Striga asiatica*. These include the substituted urea compounds thiourea and allylthiourea (Brown and Edwards, 1945) whose significance as natural stimulants is unknown. Oxidizing agents such as sodium hypochlorite, sulfuric acid, calcium hydroxide and some halogens have been tested as germination stimulants (Egley, 1972; Hsiao *et al.*, 1981). Sodium hypochlorite caused germination of *Alectra vogelii* seeds in the absence of any conditioning period, although, with conditioning, germination rose to 81–91% (Okonkwo and Nwoke, 1975). French and Sherman (1976) were also able to induce germination of *Aeginetia indica* using sodium hypochlorite. This chemical slightly stimulated the germination of conditioned and non-conditioned *Striga gesnerioides* seeds and at low concentrations acted synergistically with kinetin, ethylene and root exudate treatments (Igbinnosa and Okonkwo, 1992). The mechanism by which treatment with oxidants causes germination is unknown, but it may result from the chemical oxidation of endogenous ACC to ethylene: the *Striga* seeds stimulated with hypochlorite or sulfuric acid have a morphology similar to those stimulated with exogenous ACC (Egley, 1972; Hsiao *et al.*, 1981; Logan and Stewart, 1991). Alternatively, these treatments may stimulate germination by overcoming seed-coat-imposed mechanical inhibition of radicle growth (Barton, 1965). The results of Egley (1972) corroborate this view; he found that punctures made to the radicle end of the seed coat of conditioned seed in the absence of host-root exudate resulted in germination only slightly lower than after treatment with host-root exudate (86% compared with 98%). It has been suggested that ethylene stimulates germination by overcoming such mechanical resistance, by promoting radial-cell expansion in the embryonic hypocotyl (Abeles, 1986); thus, these scarification treatments may overcome the need for ethylene action.

Light

The effect of light on the germination of parasitic angiosperms is primarily determined by the nature of their parasitism. Root parasites of the Scrophulariceae and Orobanchaceae that germinate in the absence of host-derived stimulants, such as *Castilleja coccinea* (Malcolm, 1966), *Sopubia delphinifolia* G. (Don.) (Shivanna and Rangaswamy, 1976) and *Buchnera hispida* Buch. (Okonkwo and Nwoke, 1974), are stimulated to germinate by light. Sahai and Shivanna (1982) suggest that, since the food reserves of these small seeds are limited, they must germinate very near the soil surface to enable quick emergence and onset of photosynthesis. Parasites which require to be close to the hosts' roots to allow stimulation of germination by host-derived chemicals do not require light for germination. Germination of the seeds of *Striga asiatica* (Worsham *et al.*, 1964) and *Aeginetia indica* (French and Sherman, 1976) was inhibited by light, although germination of water-washed seeds of *Striga euphrasioides* was stimulated by daylight (Rangaswamy and Rangan, 1966). The effects of light on host-stimulated germinating seeds of *Striga asiatica* are similar to those caused by treatment with cytokinins, notably reduced extension growth of the radicle, increased diameter, production of root hairs and initiation of the haustorium (Williams, 1961b). These effects are unlikely to have any significance in nature.

Conclusions

It is apparent from searching the literature on parasitic angiosperms that little research has been carried out on those species which are of no economic or agricultural significance. However, species of agricultural significance are also those with the most advanced and unique life cycles and germination requirements. The conditioning process is not necessary for *Striga hermonthica* to germinate in response to exogenous ethylene (Logan and Stewart, 1991), but it is an absolute requirement for seeds of this species to respond to host-derived stimulants; it is not known whether this is also true of other species of *Striga* and those of *Orobanche*. Whether failure to respond to host-derived triggers is due to germination inhibition or to an inability to perceive or transduce the host signal is unknown.

Although certain germination stimulants have been identified, it is clear that they comprise only a small part of a chemical cocktail in the host/non-host rhizosphere capable of stimulating germination. It has been suggested that these germination stimulants

have a primary role as allelochemicals and that root-parasites have evolved to use these chemicals as germination triggers thereby increasing their chances of attachment and survival (Lynn and Chang, 1990). These chemicals could act in a similar way to coronatine, a toxin produced by the plant-pathogenic bacterium *Pseudomonas syringae*, which is known to stimulate ethylene production in plant tissues, with all its varied biological effects attributable to the action of this ethylene (Ferguson and Mitchell, 1985; Kenyon, 1991). It is unknown whether similar chemicals are present in host-root exudates or, indeed, whether known germination triggers such as strigol and sorgoleone-358 have pathogenicity. The allelochemical hypothesis is strengthened by the existence of germination triggers in root-exudates of hosts and non-hosts which are not indigenous to the parasite's habitat. The knowledge that germination of *Striga hermonthica*, initiated by host-derived stimulants or cytokinins results from the promotion of ethylene biosynthesis and that it is this ethylene which initiates germination suggests that the triggering of germination may be a modification of the host-wound syndrome whereby wounding is accompanied by a burst of ethylene production (Abeles, 1973).

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