Fitness of triazine susceptible and resistant *Solanum nigrum* L. in maize

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Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van de Landbouwuniversiteit Wageningen,
dr. C.M. Karssen
in het openbaar te verdedigen
op dinsdag 15 december 1998
des namiddags te half twee in de Aula

ISBN 90-5485-972-5

Stellingen

1 Alleen door alle fasen van de levenscyclus te betrekken in de analyse kunnen fitness verschillen tussen triazine gevoelige en resistente biotypes worden geïdentificeerd; bij zwarte nachtschade zijn het vooral de zaadeigenschappen die deze verschillen veroorzaken.
Dit proefschrift

2 Zaden van triazine resistente biotypes van zwarte nachtschade hebben een lagere minimum kiemtemperatuur en een minder diepe kiemrust dan zaden van gevoelige biotypes.
Dit proefschrift

3 Het verschil in fitness tussen triazine gevoelige en resistente zwarte nachtschade kan worden benut in het beheersen van triazine resistente biotypes door een toename van het aantal grondbewerkingen.
Dit proefschrift

4 Reeds vanaf de introductie van nieuwe chemische onkruidbestrijdingsmethodieken dienen strategieën ter voorkoming van resistentieontwikkeling te worden toegepast; dit geldt vooral bij grootschalige introductie van herbiciden in ontwikkelingslanden en Oost-Europa.

5 Met herbicide resistente gewassen lossen we de onkruidproblematiek niet op.

6 De mogelijkheden voor de verspreiding van resistentiegenen naar de natuurlijke omgeving zijn onvoldoende bewezen om realistische risicoanalyses te maken.

7 Het ecologiseren van de landbouw vereist herbicidenonderzoek.

8 Planten in veldproeven zijn extra aantrekkelijk voor herbivoren.

9 Een krachteloze groene lobby is slecht voor het milieu.

10 Voor een bedrijf is het van strategisch belang om ook bij een sterke marktpositie de klant als koning te blijven behandelen.

11 Het verplaatsen van Schiphol naar zee biedt nieuwe mogelijkheden voor natuurontwikkeling.

12 Financiële speelruimte zou veel meer als randvoorwaarde dan als leidraad moeten dienen bij het uitzetten van fundamenteel en strategisch onderzoek.

Stellingen behorende bij het proefschrift
"Fitness of triazine susceptible and resistant Solanum nigrum L. in maize"

Eddie Kremer
Wageningen, 15 december 1998.

ABSTRACT

Kremer E (1998) Fitness of triazine susceptible and resistant *Solanum nigrum* L. in maize. PhD thesis, Wageningen Agricultural University, Wageningen, 125 pp.

The introduction of selective herbicides has led to a rapid adoption of chemical weed control by farmers. The success of these herbicides is based on high reliability and low costs but the sole reliance on herbicides for weed control in cropping systems has resulted in a fast increase in the incidence of herbicide resistant weeds worldwide. In the case of triazine resistance, the resistance trait is linked with a fitness penalty in the absence of the selecting herbicide. Aim of this study is to assess perspectives to use this fitness penalty in the development of management strategies to control triazine resistant weed populations.

The determination of fitness requires knowledge on different life history processes. Therefore, experiments on the germination, emergence, growth, seed production, and seed longevity of triazine susceptible and resistant biotypes of *S. nigrum* in maize were performed. In germination experiments, a lower dormancy and a lower minimum germination temperature requirement of the resistant biotype was found compared to that of the susceptible biotype. The germinated seeds of the resistant biotype emerged better from different soil depths than seeds of the susceptible biotype. A growth analysis with both biotypes at low light levels under controlled conditions showed a lower final biomass production of the resistant biotype after 29 days while the relative growth rates of both biotypes in the young growth phase were similar. A lower biomass and seed production of the resistant biotype compared to the susceptible biotype was also observed in field experiments where both biotypes were grown in competition with a maize crop. Seed longevity appeared to be much greater for susceptible seeds than for resistant seeds during the summer, while in the winter, differences in seed longevity were minimal.

The integration of the different life history processes of both biotypes into a population dynamics model showed a strongly reduced fitness of the triazine resistant biotype compared to the susceptible biotype of *S. nigrum* in an agro-ecosystem with continuous maize cropping. The performance of the resistant biotype was in most of the life history processes inferior to that of the susceptible biotype which in combination resulted in a strong fitness reduction. The relative fitness of the resistant biotype compared to that of the susceptible biotype was 0.65. The most important determinant of the observed fitness difference between susceptible and resistant plants was the difference in seed characteristics.

The simulation model showed the importance of measures to reduce selection pressure for the development of a triazine resistance management strategy. Management strategies exploiting the observed fitness differences between triazine susceptible and resistant biotypes of *S. nigrum* in the continuous cropping of maize should be aimed at exhaustion of the seed reserve of the resistant population in the seed bank.

Key-words: *Solanum nigrum*, black nightshade, maize, triazine resistance, fitness, life history, population dynamics model, resistance management

Voorwoord

Met dit proefschrift is een einde gekomen aan 6 jaar onkruidkundig onderzoek. Gedurende die 6 jaren is duidelijk gebleken dat je zoiets niet in je eentje doet. Dit lijkt dan ook de juiste plaats en tijd om alle mensen die in meerdere of mindere mate aan mijn onderzoek hebben bijgedragen te bedanken.

Allereerst wil ik Henk Naber noemen die het project geïnitieerd heeft. Gedurende de eerste fase van het project op de vakgroep VPO heb ik prettig samengewerkt met Jaap Blijjenberg en René Siep in de verschillende proeven. Nadat de groep Onkruidkunde was overgeplaatst naar TPE heb ik veel hulp gehad van verschillende mensen bij Unifarm, die erg veel werk verzet hebben in mijn proeven zowel binnen als in het veld. Hiernaast heb ik voor de proef behorende bij hoofdstuk 3 hulp gehad van Jos Kroesbergen van de vakgroep Grondbewerking.

Nadat de experimentele resultaten waren verzameld werden die geanalyseerd met Jacques Withagen, gerapporteerd, en uitgebreid bediscussieerd in het promovendi-overleg met mijn collega-AIO's. Bij het schrijven van mijn artikelen kreeg ik specialistische begeleiding van Bert Lotz en op de achtergrond Paul Struik. Gon van Laar heeft veel nuttige adviezen gegeven bij het produceren van dit proefschrift. Een speciaal woord van dank is op zijn plaats voor Martin Kropff die bij zijn aanstelling als prof. Onkruidkunde heel wat werk heeft moeten verzetten om de zaak weer goed op de rails te krijgen. Met gezamenlijke inzet, ook buiten kantooruren, is dit project nu afgerond en is er zelfs een simulatiemodel in mijn proefschrift opgenomen.

Naast de inhoudelijke kant was de sociale kant toch ook minstens zo belangrijk om prettig te kunnen werken. Meest intensief heb ik natuurlijk opgetrokken met mijn kamergenoten waarbij ik in de laatste drie jaar met Leo veel humorvolle momenten heb mogen meemaken. Daarnaast hebben ook mijn andere collega's het nodige moeten verduren tijdens koffie- en lunchpauzes; ik hoop dat jullie het net zo leuk hebben gevonden als ik. Natuurlijk zijn er nog veel meer mensen die op enige wijze hebben bijgedragen aan de voltooiing van dit proefschrift en die ik niet allemaal kan opnoemen, jullie ook bedankt.

Buiten de reguliere werktijden heb ik veel steun mogen krijgen van Ineke, zowel in de vorm van afleiding als die van hulp bij het uitvoeren van proeven met name in het weekend; jammer dat de samenwerking niet voortduurt. Als laatste wil ik nog mijn familie bedanken, helaas mocht mijn moeder alleen de uitvoering maar niet de succesvolle afronding van mijn proefschrift meemaken. Pap, Gert, Wim en Marianne, bedankt dat jullie nooit genoeg kregen van mijn "werkverhalen" en steeds bij mijn werk betrokken zijn gebleven.

Eddie

ACCOUNT

Parts of this thesis have been included in the following publications:

Chapter 2	Kremer E & Lotz LAP (1998) Germination and emergence characteristics of triazine-susceptible and triazine-resistant biotypes of *Solanum nigrum*. *Journal of Applied Ecology* **35,** 302-310.
Chapter 3	Kremer E & Lotz LAP (1998) Emergence depth of triazine susceptible and resistant *Solanum nigrum* seeds. *Annals of Applied Biology* **132,** 277-288.
Chapter 4	Kremer E & Kropff MJ (xx) Comparative growth of triazine susceptible and resistant biotypes of *Solanum nigrum* at different light levels. *Annals of Botany* provisionally accepted.
Chapter 5	Kremer E & Kropff MJ (xx) Growth and reproduction of triazine susceptible and resistant *Solanum nigrum* in a maize crop. *Weed Research* in press.
Chapter 6	Kremer E (xx) Seed survival of triazine susceptible and resistant *Solanum nigrum.* submitted.
Chapter 7	Kremer E & Kropff MJ (xx) Population dynamics of triazine susceptible and resistant *Solanum nigrum* in maize: a simulation analysis. submitted.

CONTENTS

Chapter 1

General introduction

Herbicide resistance in weeds

Selective herbicides were introduced in agriculture about 50 years ago and became very successful because of the high reliability and the low costs. The sole reliance on herbicides for weed control in different cropping systems has resulted in a fast increase in the number of herbicide resistant weeds worldwide. Herbicide resistance of weeds is here defined as the naturally occurring inheritable ability of some biotypes within a weed population to survive a herbicide treatment that would, under normal conditions of use, effectively control that weed population (Heap 1997).

The first reports on resistant weeds concerned triazine resistant *Senecio vulgaris* L. in a nursery in 1968 where simazine was used once a year for 10 years (Ryan 1970). Within the following years, triazine resistance evolved rapidly (Fig. 1.1). In 1995, 43 dicotyledonous and 18 monocotyledonous weed species had evolved resistance to triazine herbicides worldwide. The majority of triazine resistant weeds has been identified in maize production areas in North America and Europe and in orchards in Europe. Based on a survey, it is estimated that over three million hectares are infested with triazine resistant weeds worldwide, making them the most widespread resistance problem (Heap 1997). Other important herbicide groups to which resistance has been observed are the ALS inhibitors, the bipyridiliums, the ureas/amides, the synthetic auxins, and the ACCase inhibitors (Heap 1997).

Herbicide resistant weeds are often controlled by the use of alternative herbicides or mixtures of herbicides with different modes of action. However, this strategy may appear to be ineffective because of cross-resistance and multiple resistance. Cross-resistance is where a biotype is resistant to two or more herbicides due to the presence of a single resistance mechanism. Multiple resistance is where the resistant biotype possess two or more distinct resistance mechanisms. Since this means that alternative herbicides might not always be the ultimate solution, other management strategies to control resistant weeds should be developed as well.

Management strategies to delay and/or prevent the occurrence of herbicide resistance can be based on two biological processes: alteration of selection pressure and back-selection (Cousens & Mortimer 1995). If the herbicide resistance trait does not come with a fitness penalty in the absence of the selecting herbicide, reduction of selection pressure is the most important element of herbicide resistance management. Changes in selection pressure of chemical weed control measures may be achieved by reduction of the dosage, reduction of herbicide persistence, switching to herbicides with different modes of action, using herbicide mixtures, or a rotation of herbicides with different modes of action over cropping seasons (Rotteveel *et al.* 1997). In the case of propanil resistant *Echinochloa crus-galli* L. in rice, this resulted in a suggested strategy which included rotating crops when possible, using tillage practices when

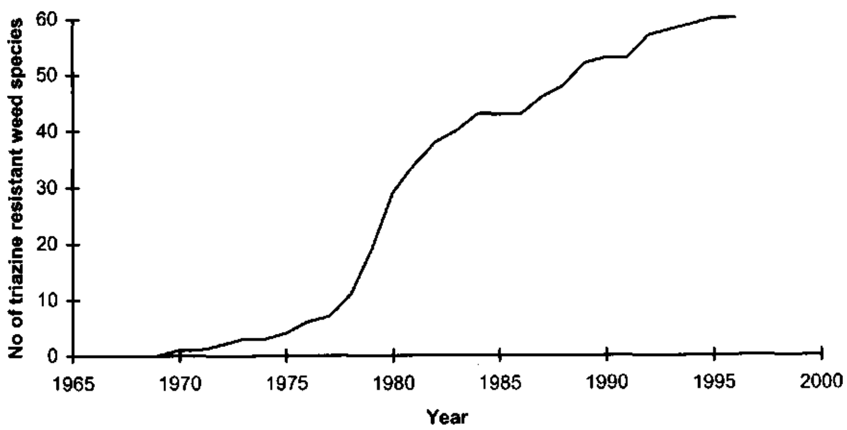

Fig. 1.1 The increase in number of triazine resistant weed species worldwide.

possible, rotating herbicides with different modes of action, avoiding sequential application of the same herbicide or herbicides with the same mode of action, and application of mechanical control of weeds when fields are fallow (Baldwin *et al.* 1995). In Australia, *Lolium rigidum* has become resistant to many herbicide classes which forced the adoption of Integrated Weed Management strategies. The IWM strategies to control herbicide resistant *L. rigidum* include various combinations of factors including pasture and crop rotations, variation in seeding date, use of non-selective herbicides, high crop seeding rates, vigorous crop growth and capture of weed seed in the harvest operation (Powles *et al.* 1997).

The use of back selection in a resistance management strategy is possible when there is a fitness difference between biotypes in absence of the selecting herbicide. The fitness of a biotype is defined by its survival and reproduction which is determined in the context of environmental conditions and other biotypes in that environment (Holt & Thill 1994). The often assumed reduced fitness of the resistant biotype compared to that of the susceptible biotype in the absence of the selecting herbicide may be further reduced by competition or other environmental factors under the control of farm managers (Cousens & Mortimer 1995).

Triazine resistant S. nigrum in maize

The development of IWM strategies based on back-selection requires detailed insight in the differences of life history processes separately and in combination. This study aimed to study these aspects in an integrated way in one cropping system-weed-herbicide combination. The relatively simple system with triazine susceptible and resistant *S. nigrum* in maize was selected because triazine resistant biotypes of *S. nigrum* in maize are widespread in The Netherlands and triazine resistance is the most well documented case of resistance (Warwick 1991, Holt & Thill 1994, Warwick & Black 1994, Jasieniuk *et al.* 1996). The maize crop is grown continuously in the same field with the same cropping practice every year. This gives a very constant and stable environment to study the dynamics of triazine susceptible and resistant biotypes of *S. nigrum* in depth.

In the 1950s, herbicides from the triazine group were introduced for weed control in different crops. The introduction started with simazine for the control of weeds in nurseries and orchards in 1956, followed by atrazine, mainly used for weed control in maize, in 1958 (Kirkwood 1987). Triazine herbicides inhibit the electron transport between Q_A and Q_B in photosystem II in the chloroplasts (Holt *et al.* 1993). The herbicide not only works as a contact herbicide, but also has a long lasting soil activity which gives opportunities for control of late emerging weeds such as *S. nigrum*. Disadvantages of the lasting soil activity of triazines are the possible damage in a susceptible crop grown afterwards, the contamination of groundwater, and the high selection pressure put on the weeds.

In The Netherlands, triazine resistant weeds were not discovered until 1982. Van Dord (1982) found atrazine resistant *Chenopodium album* L. in maize fields and simazine resistant *Poa annua* in fruit orchards. In 1985 and 1986, Van Oorschot and Straathof (1988) assessed the occurrence of triazine resistant weeds in soil samples in The Netherlands. Triazine resistance was most frequently observed in *C. album* and *S. nigrum* from maize fields.

Maize (*Zea mays* L.) has been known as a forage crop in The Netherlands since the 1930s. Starting in 1970, the area cropped with silage maize increased very rapidly up to ± 11% of the cropping area in The Netherlands (Fig. 1.2). Silage maize is mainly grown at dairy farms on sandy soils. The crop is successful because it is easy to cultivate, produces large quantities of high quality forage, the produce is easy to conserve, the crop tolerates manure application in large quantities, cropping practices can mainly be done by contractors, and the field does not have to be close to the farm. The crop is sown at the end of April or the beginning of May because of the high minimum temperature for growth. Most silage maize is grown in continuous cropping with the same cropping practice every year.

Weed control in maize is mainly performed with herbicides although the use of mechanical weed control methods increases. Weed control with soil-applied herbicides is easy to perform, does not require a lot of labour, and does not require a precise timing of application. The three major dicotyledonous annual weeds in maize in The Netherlands are *Polygonum persicaria* L., *C. album*, and *S. nigrum*. The continuous use of soil applied herbicides from the triazine group in the continuous cropping of maize resulted in the development of triazine resistant *C. album* and *S. nigrum* (Van Oorschot & Straathof 1988). The uncontrolled *S. nigrum* population might not only compete with the maize crop in the early growth stages but there is also a risk of detrimental effects on animal health through the poisonous berries.

The species *Solanum nigrum* L. is endemic in temperate and tropical zones. Nowadays it is a common and often notorious weed in both agricultural and horticultural crops in most parts of the world. In The Netherlands, seeds germinate

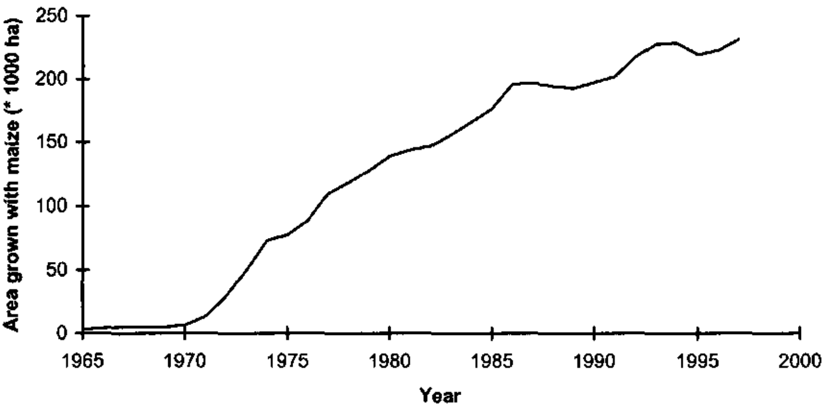

Fig. 1.2 The increase in area grown with maize in The Netherlands in time (Anonymous 1965-1997).

relatively late in the season (late April - July) because of a high minimum temperature requirement (Weller & Phipps 1979, Givelberg & Horowitz 1984, Van den Brand 1985, Mahn & Lemme 1989) over a considerable time span (Weller & Phipps 1979). Secondary dormancy is induced by high soil temperatures (Roberts & Lockett 1978). The sympodial growth of *S. nigrum* results in a very high seed production which only stops when the plants are killed by night frost or harvest operations. The autogamous reproduction of *S. nigrum* allows for a rapid build-up of populations from a few individuals (Basset & Munro 1985).

The seeds of *S. nigrum* can stay viable in the soil seed bank for many years (Roberts & Lockett 1978). Seeds removed from the field in the harvest product spread easily by contamination of seed crops, or in manure after ensiling and passage through the alimentary tract of many animals (Weller & Phipps 1979, Elema & Scheepens 1992). High densities of *S. nigrum* in the silage maize can be harmful for animals due to the presence of toxic alkaloids (solanine and solanidine) and high nitrate levels (Weller & Phipps 1979, Vogel & Gutzwiller 1993). To reduce competition of *S. nigrum* with the crop and to prevent the risk of detrimental effects on animal health, complete control is necessary including control of triazine resistant biotypes of *S. nigrum.*

In *S. nigrum,* the triazine resistance mechanism is based on a modification of the target site which is caused by a mutation in the chloroplast *psbA* gene and maternally inherited. The mutation decreases the affinity of Q_B for triazines and also reduces the electron transport rate between Q_A and Q_B in photosystem II (Holt & Thill 1994). Beside this most common form, in a few reported cases, triazine resistance was based on enhanced metabolism of the herbicide (Gronwald *et al.* 1989).

Triazine resistant biotypes are present in weed populations in varying, but low, frequencies before any exposure to herbicides (Moss & Rubin 1993). Gressel (1991) estimated an initial frequency of 10^{-10} to 10^{-20} triazine resistant biotypes in nonexposed populations but in some weed populations the initial frequency of triazine resistance must have been considerably higher based on herbicide management history of the field.

Triazine resistance management based on fitness differences

The concern about the adverse environmental effects of chemical control has resulted in legislation that restricts the amount of pesticides used in Dutch agriculture. Besides the aim to reduce the amount of pesticides used in agriculture by 45% in 2000 compared to the reference period 1984-1988 (Anonymous 1991), dependence on herbicides also has to be reduced.

In practice, triazine resistant weeds are mainly controlled with the use of alternative herbicides or mixtures of different herbicides. This does not comply with the objectives of the Dutch government. In field experiments, triazine resistant weeds were controlled by herbicide mixtures which included dicamba, bromoxynil, 2,4D, alachlor, or metolachlor (Birschbach *et al.* 1993, Parks *et al.* 1996). Triazine resistant *S. nigrum* showed an increased susceptibility ("negative cross-resistance") to bentazone and pyridate (Van Oorschot & Van Leeuwen 1988, Claux *et al.* 1992, Arlt & Jüttersonk 1992, Parks *et al.* 1996). This means that triazine resistant weeds are successfully controlled but the amount of herbicides and the costs increase compared to situations without triazine resistance. The increase in herbicide use and the continuing dependence on herbicides does not agree with the Dutch policy. Therefore, ecologically-based weed management strategies should become the most important factor in the control of triazine resistant weeds.

Besides the increase in herbicide use, cross-resistance to other herbicides will make these herbicides useless for weed control in fields containing triazine resistant weeds. Depending on the weed species, triazine resistant weeds may be cross-resistant to herbicides of the chemical groups of carbamates (Solymosi & Lehoczki 1989), uracils (Fuerst *et al.* 1986), or urea's (Burnet *et al.* 1991). The development of multiple resistance is another reason why triazine resistant weed populations should be properly controlled (Matthews 1994).

Triazine resistance seems to be the only case where the resistance trait comes with a reduced fitness and where back-selection is a serious option for the management of resistant biotypes. This would reduce the amount of herbicides used and also reduce the reliance on herbicides. It is suggested that the less efficient electron transport, arising from the mutation causing resistance, results in reduced vigour and overall fitness of the resistant biotype compared to the susceptible biotype in the absence of the herbicide (Cousens & Mortimer 1995). The fitness component survival may be further subdivided into success of seed, seedling, and mature plant which in turn are functions of processes such as seed persistence, germination, dormancy, establishment, and growth. Reproductive success is determined by pollen and seed production. The estimation of relative fitness of susceptible and resistant biotypes is difficult because of three problems: the choice of material for comparison, the experimental conditions under which the fitness is compared, and the plant characteristics used to measure fitness (Jasieniuk *et al.* 1996).

Early research about fitness differences between biotypes often included comparisons between biotypes from different geographical origins with different genetic backgrounds (e.g. Warwick & Marriage 1982). The different nuclear genome controlled traits may compensate to some extent the secondary effects of triazine resistance (Holt & Thill 1994). Therefore, nuclear isogenic lines should be used to study the effect of the chloroplast mutation separately from other genetic differences (Warwick 1991). However, Holt (1996) argued that the relevance of fitness comparisons to develop management strategies should include field selected susceptible and resistant biotypes since isogenic biotypes do not occur naturally in the field. In that case, comparisons between susceptible and resistant biotypes from the same field strongly improve the validity of the conclusions.

Since fitness depends on the environmental conditions, measurements of fitness to understand the rate of resistance evolution or management of resistance should be determined under field conditions with a relevant crop and with and without herbicide application (Roush *et al.* 1990). Basic processes e.g. growth of susceptible and resistant biotypes might be studied under controlled conditions but results should always be validated under field conditions (Jordan 1996).

The most common characteristics used to measure fitness are biomass production and accompanying seed production. In general, the triazine susceptible biotype had an equal or higher fitness than the resistant biotype when these parameters were examined (Warwick 1991, Warwick & Black 1994, Holt & Thill 1994, Jasieniuk *et al.* 1996). Studies comparing isogenic lines indicate that resistant biotypes are either less fit, or as fit as susceptible biotypes (Jasieniuk *et al.* 1996). However, not only vegetative and reproductive growth, but other life history processes also determine the fitness of a biotype.

Reports on germination characteristics of triazine susceptible and resistant biotypes gave variable results. At optimal temperatures, similar germination responses of susceptible and resistant were found in *Amaranthus retroflexus* L. (Weaver & Thomas 1986) and *S. nigrum* (Bulcke *et al.* 1985, Gasquez *et al.* 1981). A higher total germination of the susceptible biotype was observed in *Amaranthus powellii* S. Wats. (Weaver & Thomas 1986) and *Brassica campestris* L. (Mapplebeck *et al.* 1982). A higher total germination of the resistant biotype compared with the susceptible biotype was found in *C. album* (Warwick & Marriage 1982) and *Phalaris paradoxa* L. (Schönfeld *et al.* 1987). The emergence of germinated seeds from a certain soil depth was better for the triazine susceptible biotype of *B. campestris* (Mapplebeck *et al.* 1982) and better for the triazine resistant biotype of *P. paradoxa* (Schönfeld *et al.* 1987). In one of the rare cases where seed longevity of triazine susceptible and resistant biotypes were studied, Watson *et al.* (1987) found that the resistant biotype of *Senecio vulgaris* L. had a greater seed longevity in the lower seed bank than the susceptible biotype. In the surface seed bank (0-2 cm deep), seed longevity of both biotypes depended on management. The variable results of different experiments with different species emphasize that fitness must be measured over the whole life cycle to encompass the effect of selection on mortality and fecundity of survivors. Existing population dynamics models show the importance of the evaluation of a number of life history stages such as dormancy, germination, establishment, survival, growth, pollination, and seed production too (Maxwell *et al.* 1990).

In order to manage triazine resistance with back-selection, it is imperative that the relative fitness of triazine susceptible and resistant biotypes in the absence of herbicide selection pressure is better understood (Holt & Thill 1994). A reduced fitness of the resistant biotype in the absence of selection by the herbicide allows alternative control strategies to exploit this weakness (Cousens & Mortimer 1995). Available control strategies may include an effective herbicide use, exploitation of unique aspects of the biology of the weed species and its biotypes, and manipulation of the cropping system to maximize both chemical and non-chemical control of both biotypes.

Simulation models

Models for different life history processes have been developed such as emergence (Vleeshouwers 1997) and crop-weed interactions (Kropff & Van Laar 1993). These models are useful for analysis of parts of the life cycle. However, the integration of genetic, ecological, and physiological aspects involved in the evolution and management of herbicide resistance requires the development of simulation models that contain all major life history processes. These models allow to assess the relative importance of factors controlling the development and spread of herbicide resistance inherited on one or a few major genes (Moss & Rubin 1993). Hardly any studies on all aspects of the life cycle have been performed but in this thesis the fitness of triazine susceptible and resistant biotypes is analysed with experimental data on the most important components of the life cycle. The models of May and Dobson (1986) and Gressel and Segel (1990) estimated the increase of the fraction resistant biotypes in the population based on the initial frequency of the resistant biotype, the selection pressure imposed by the herbicide, the relative fitness of resistant biotypes compared to susceptible biotypes, and the longevity of seeds in the seed bank. Maxwell *et al.* (1990) separated two major processes determining the dynamics of herbicide resistance, one being fitness of the resistant biotype compared to the susceptible biotype and the other one being gene flow in space and time. The four key components in the development of herbicide resistance in the population arising from these theoretical models are selection pressure, initial frequency of the resistance gene, seed persistence in the seed bank, and the per capita rate of increase of both biotypes (Mortimer *et al.* 1992).

A theoretical model which included experimental data was developed for chlorotoluron resistance in *Alopecures myosoroides* Huds. by Mortimer *et al.* (1992). Selection coefficients were calculated from experimental data regarding the application of chlorotoluron to susceptible and resistant *A. myosoroides* grown at a range of densities and frequencies in presence of a winter wheat crop. The selection coefficient was used to model changes of the resistance allele frequency, further based on crop rotation, survival of seeds in the soil and survival of mature plants. Simulation results showed the importance of the interaction between density-dependent and density-independent regulation on the resistance allele frequency and showed that in herbicide rotations the magnitude of density-independent regulation may be critical to the success of herbicide rotations as part of a management programme.

Zanin and Lucchin (1990) determined the reproductive fitness of triazine susceptible and resistant biotypes of *S. nigrum* in competitive conditions. This fitness parameter was used in the simulation of the evolution of the resistance frequency in the population depending on crop rotation and selection pressure of different weed control strategies. Their simulation results emphasized the importance of selection pressure and herbicide choice in a crop rotation as potential components of herbicide resistance management strategies.

A major shortcoming of these studies is that when experimental data were used to parameterize theoretical models, only the seed production per plant was included as fitness parameter. The development of a resistance management strategy based on ecological characteristics using the fitness differences between susceptible and resistant biotypes requires the integration of parameters based on real data on different life history processes. The study of the effect of fitness of biotypes on the population dynamics in a crop should involve the use of a population dynamics model what is parameterized using experimental data on all life history processes. In this way, sensitivity studies show the relative importance of the differences found between susceptible and resistant biotypes in the different processes and scenario studies are more complete and may be more valid and closer to reality resulting in the development of realistic management strategies to prevent or control triazine resistance. This study aimed to conduct such a complete modelling study based on experimental data on all life history processes for one crop-weed-herbicide combination.

Objectives and outline of the study

The aim of this study was to determine fitness differences between triazine susceptible and resistant biotypes of *S. nigrum* in a maize crop. Fitness differences were analysed by studying different life history processes of both biotypes from different populations in the absence of the selecting herbicide. The obtained knowledge was integrated into a population dynamics model. This model was used for sensitivity analysis and scenario studies to explore how the observed fitness differences between biotypes can be exploited to manage triazine resistant weeds by changing management practices without an increase in herbicide use.

In Chapter 2, the emergence patterns of both biotypes in the field are described and linked with results on germination and dormancy characteristics of triazine susceptible and resistant seeds of *S. nigrum*. Possibilities for weed control with an extra early soil cultivation are discussed. Chapter 3 describes the pre-emergence growth of germinated seeds depending on temperature and seed weight. Possible differences in maximum emergence depths between biotypes might result in different fractions of the seed bank that will successfully emerge. In Chapter 4, the basic growth processes of susceptible and resistant biotypes from five different populations in the young growth phase were studied in the growth chamber at different limiting light levels. Biomass and seed production of both biotypes were also studied in the field. The effect of a maize crop on the growth parameters of both biotypes is described in Chapter 5. In Chapter 6, the survival of susceptible and resistant seeds in the seed bank during the year is described. In Chapter 7, the experimental data from the different chapters are integrated in a model for the dynamics of populations of susceptible and resistant biotypes. Scenario studies were used to explore the possibilities for successful management of triazine resistant *S. nigrum* in maize. In the general discussion, the conclusions from the experiments and the model are discussed.

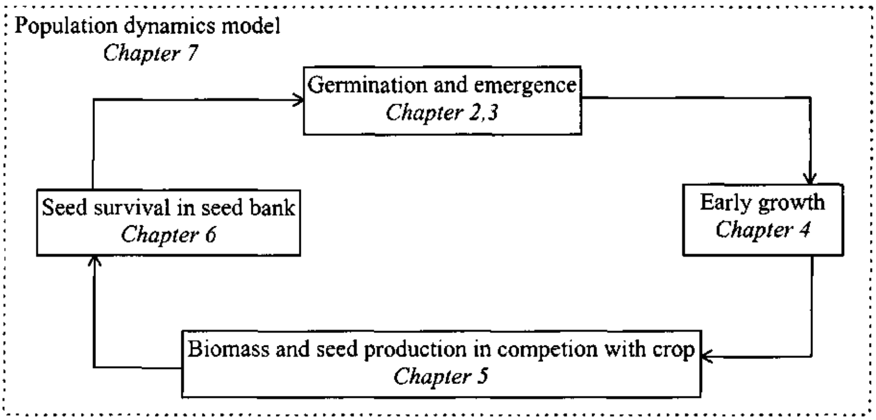

Fig. 1.3 Schematic representation of the contents of the thesis.

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Chapter 2

Germination and emergence characteristics of triazine susceptible and resistant biotypes of *Solanum nigrum*

Abstract

Seedling emergence patterns of triazine susceptible and resistant *Solanum nigrum* in the field were studied in Wageningen, The Netherlands. Emergence patterns were similar in the first year, but in the second year resistant seedlings emerged faster and the number of resistant seedlings was higher.

To explain emergence patterns, a germination experiment was carried out. Seeds from two populations with triazine susceptible and resistant biotypes were buried in late autumn and exhumed monthly during spring. Germination was assessed in incubators at different constant temperatures. The lowest temperatures for germination of seeds from the Achterberg population ranged from 20 °C on 1 February to 10 °C on 1 May for the susceptible biotype, and from 15 °C on 1 February to 10 °C on 1 May for the resistant biotype. The lowest temperatures for germination of seeds from the Zelhem population ranged from 25 °C on 1 February to 10 °C on 1 May for the susceptible biotype, and from 15 °C on 1 February to 10 °C on 1 May for the resistant biotype. The minimum germination temperature of seeds from the resistant biotype appeared to be lower than that of the susceptible biotype.

Emergence patterns in the field could be explained by soil temperature and different minimum germination temperature requirements of seeds from the triazine susceptible and resistant biotype. This knowledge can be used to manage triazine resistant *S. nigrum* by the timing of soil cultivation.

Key-words: black nightshade, dormancy, resistance management, seed size, triazine resistance.

Introduction

Triazine resistant *Solanum nigrum* L. is becoming an increasing problem in silage maize in Europe. Resistant plants survive herbicide applications, compete with the crop, and may contaminate the harvest with poisonous berries. Herbicide resistance is here defined as an evolved tolerance in a weed population in response to selection through application of a herbicide (Cousens & Mortimer 1995).

A key factor in the development of herbicide resistance in the field is the seed bank. The importance of a seed bank in evolutionary terms is that it represents a "memory" of past selection events which may buffer evolutionary processes and may serve to delay the onset of resistance (Templeton & Levins 1979). Gressel and Segel (1990) also reported on the importance of seed longevity and dormancy to the dynamics of resistant weed populations. Differences in viability, dormancy and germination characteristics may have major implications in the management of fields infested with herbicide resistant weed populations and the persistence of these populations in the field.

Depleting the seed bank by reducing dormancy or stimulating germination may enhance the management of weeds in general (Dyer 1995, Johnson & Mullinix 1995). Differences in germination and dormancy between resistant and susceptible weeds, resulting in different emergence patterns, could be exploited to manage herbicide resistant weed populations. If resistant seedlings emerge earlier than susceptible seedlings, an extra control measure prior to crop sowing will result in a greater reduction of the resistant population. This has also been suggested for the control of earlier germinating sulfonylurea resistant *Kochia scoparia* (Thompson *et al.* 1994).

Differences in germination between triazine susceptible and resistant *S. nigrum* have been reported. Gasquez and colleagues (1981) found that the fraction of triazine resistant *S. nigrum* that germinated at different alternating temperatures up to 25 °C was lower than that of susceptible biotypes. In a study by Bulcke *et al.* (1985), resistant and susceptible biotypes of *S. nigrum* from different geographic origins showed similar germination at 20 °C. A mechanistic explanation for differences in the pattern of germination between resistant and susceptible biotypes might be based on a combination of two selection processes: earlier germination and emergence of resistant seedlings will increase seed production in a competitive crop, and survival and thus seed production of susceptible plants will be higher later in the season when triazine levels in the soil may have decreased to a non-lethal level.

Once dormancy of seeds is removed, factors controlling germination are, amongst others, temperature and individual seed weight. In many species a positive effect of temperature on germination has been found (Bewley & Black 1994). In general, germination increases until an optimum temperature above which germination is restrained. Speed of germination is also temperature dependent. Seed weight affects germination, viability and dormancy and varies under the influence of maternal environmental and genetic effects (Wulff 1995). For example, smaller seeds of *Rumex* have been observed to have a lower germination fraction than larger seeds (Cidecyan & Malloch 1982) while no difference has been observed amongst seeds of different size of *Solanum ptycanthum* (Hermanutz & Weaver 1991). The rate of germination was, however, lower amongst smaller seeds of *S. ptycanthum* (Hermanutz & Weaver 1991) and higher amongst smaller seeds of *Erodium brachycarpum* (Stamp 1990). Preliminary investigations showed that in some populations at least seed weight of triazine susceptible biotypes may be higher than that of resistant biotypes.

The objectives of this study were: 1) to separate the effect of biotype and seed weight on germination characteristics of *Solanum nigrum*, 2) to determine the germination fraction and speed of germination of triazine resistant and susceptible *S. nigrum* in order to explain the observed emergence patterns in the present study of susceptible and resistant biotypes in the field and 3) to indicate whether the observed emergence patterns of resistant and susceptible biotypes may have potential for the ecologically based management of herbicide resistant weed populations.

Materials and methods

The species

Solanum nigrum L. is a self-pollinating species. It is an important weed in arable field crops in temperate and tropical regions. Germination of *S. nigrum* is promoted by light and soil temperatures above 15 °C (Van den Brand 1985). Germination can be enhanced with a cold pre-treatment or application of nitrate or gibberelic acid (Roberts & Lockett 1978). In the field, *S. nigrum* has a seasonal emergence pattern: it germinates in The Netherlands between April and August because of the high minimum soil temperature needed for germination. Van den Brand (1985) reported a minimum soil temperature of 15 °C and Keeley and Thullen (1983) of 17 °C.

Triazines are the most common herbicide group used for weed control in maize in The Netherlands. Triazine resistance in *S. nigrum* consists of a single gene mutation in the chloroplast. The relative frequency of the resistance gene in natural populations without triazine applications ranges between 10^{-6} and 10^{-10}. The resistance characteristic is maternally inherited (Jasieniuk & Maxwell 1994). Resistant plants can survive triazine doses up to 30 times the regular dose while the selection pressure of a triazine herbicide is very high, killing over 98% of the susceptible plants. Several authors suggested that there is a cost in fitness terms related to this mutation, i.e. reduced photosynthesis (Holt 1997).

Emergence study

Triazine resistant and susceptible *S. nigrum* plants within one population were found in a field in Achterberg (51°59' N, 5°35' E). Plants were collected and grown in a glasshouse in 1992 for seed production. Seeds were extracted from berries by washing and sieving after which they were dried and stored at room temperature. In November 1992, batches of 4000 seeds were buried in nylon bags in the field at a depth of 5 cm. Average dry thousand kernel weight of susceptible seeds was 0.906 g and of resistant seeds 0.814 g. In spring of 1993, 10 PVC cylinders with a diameter of 30 cm were buried in the soil to a depth of 10 cm. The soil was removed and X-ray radiated with 1 MRad to kill all seeds present. To imitate soil cultivation in a maize cropping system, the over-wintered batches of 4000 seeds were on 4 May, each separately, thoroughly mixed with soil from one PVC cylinder and put back in the cylinder in the field. This resulted in a seed density in the PVC cylinder of 56588 seeds m^{-2} per 10 cm depth. Experimental set-up consisted of a randomized complete block design with 5 replicates; each replicate consisted of one cylinder with susceptible seeds and one cylinder with resistant seeds. From May until mid-August 1993, the number of emerged seedlings of *S. nigrum* was counted every 3 to 5 days. After counting, emerged seedlings were removed. In October, just before maize harvest, soil from all cylinders was removed and separately stored at 5 °C.

The same soil was again laid out in PVC cylinders in the field on 6 May 1994 and emergence of triazine resistant and susceptible *S. nigrum* was again observed for more than three months. The number of seedlings m^{-2} in each cylinder was in both years best characterized by a double Gompertz curve and a binomial distribution of the response variable:

$$y = C_1 \times e^{-e^{-B_1(x - M_1)}} + C_2 \times e^{-e^{-B_2(x - M_2)}} \qquad \text{(Eq. 2.1)}$$

In this model, y is the number of seedlings m^{-2} at time x. C_1 and C_2 are asymptotes and B_1 and B_2 are shape parameters. M_1 and M_2 represent the x-value of the inflection points where emergence rate is maximal, $1/M_1$ is used as a measure of the emergence rate (day^{-1}). Analysis of variance determined whether differences in parameters between biotypes existed. The total number of seedlings was analysed using a generalized linear regression model with a binomial distribution (Payne *et al.* 1987).

Germination study

To explain observed differences in field emergence patterns between susceptible and resistant biotypes, a study was carried out to determine the germination characteristics of both biotypes in relation to individual seed weights and temperature. In September 1995, ripe berries were harvested from *S. nigrum* plants grown in an experimental field under similar environmental conditions with no inter- or intra-specific competition. Plants originated from two maize fields with both biotypes present in each field (Achterberg (51°59' N, 5°35' E) and Zelhem (51°59' N, 6°18' E)).

Triazine resistance of plants was confirmed using measurements of quantum yield based on the knowledge that resistant plants have a lower photosynthetic activity (Dominguez *et al.* 1994). Berries were collected from 10 to 19 plants per biotype. Seeds were washed, sieved and dried at room temperature. Dry seeds from each biotype and population were divided into three batches with equal numbers of seed and different seed weights attained by separation with air, using a South Dakota blower. Individual seed weights per batch and their standard errors were determined by weighing 200 single seeds (Table 2.1). Average seed weight of the resistant biotype of the Achterberg population was significantly lower than that of the susceptible biotype. The Zelhem population showed no significant differences in average single seed weights between both biotypes. In both populations the variation in seed weights of the susceptible biotype was greater. Experimental set-up consisted of a randomized design with the treatments population, biotype, seed weight, exhumation date and germination temperature in two replicates.

On 22 December 1995, 48 nylon bags per seed batch, filled with a mixture of soil and 100 seeds of that specific batch were buried in the field at a depth of 5 cm. As a control, seeds were also dry stored at room temperature. In January, the dry stored seeds were laid out in petri dishes on moist filter paper to germinate in incubators with constant temperatures of 5, 10, 15, 20, 25 and 30 °C (12 h light). Seeds were assumed to have germinated when the radicle was visible. Germination was recorded every day.

Seeds, buried in the field, were dug up at monthly intervals on 1 February, 1 March, 1 April and 1 May 1996. Seeds were washed from the soil at 15 °C and germinated at the same 6 constant temperatures. Total numbers of germinated, apparently viable and dead seeds were recorded for every treatment at each exhumation date. The data were analysed by a stepwise multiple generalized linear regression with a binomial distribution (Payne *et al.* 1987) where the total fraction germinated was defined as the number of germinated seeds divided by the total number of viable seeds. Factors in the regression model were exhumation date, population, biotype, temperature and seed weight. Cumulative germination fraction in time (z) was best characterized by a Gompertz curve with a binomial distribution of the response variable:

$$z = C \times e^{-e^{-B(x-M)}} \qquad \text{(Eq. 2.2)}$$

In this formula, x is time, M is a time scale constant, B is a shape parameter, and C represents the asymptote. The formula was used to determine the t_{50}, which represents the time at which 50% of the total germination fraction has germinated. Germination rate was expressed as $1/t_{50}$ (day^{-1}) and analysed within the temperature range where a sufficient number of seeds had germinated. Analysis was performed with a stepwise multiple linear regression with a normal distribution (Payne *et al.* 1987).

Table 2.1. Mean individual seed weights of *S. nigrum* of the different sized fractions of the biotypes from different populations.

Population	Biotype	Fraction	Seed weight (10^{-6} g) with standard error in parentheses
Achterberg	Susceptible	I	784.8 (7.61)
		II	805.4 (6.46)
		III	879.7 (6.71)
	Resistant	I	727.6 (6.71)
		II	751.6 (6.75)
		III	793.9 (6.28)
Zelhem	Susceptible	I	677.3 (8.68)
		II	759.2 (7.96)
		III	820.0 (7.72)
	Resistant	I	745.5 (7.05)
		II	785.1 (6.96)
		III	800.3 (5.97)

Results

Emergence study

There were no differences in emergence patterns of the resistant and susceptible biotypes from the Achterberg population in 1993 (Fig. 2.1). The onset, emergence rate and total number of emerged seedlings were similar. The total number of emerged seedlings represented about 45 % of the number of seeds within the potential emergence depth of 5 cm. In 1994, the differences in the emergence pattern between both biotypes were very clear. The total number of emerged resistant seedlings was significantly higher than the total number of susceptible seedlings ($P<0.05$). Emergence rate of the resistant seedlings was also significantly higher than that of the susceptible seedlings ($P<0.05$). To further interpret emergence patterns, average daily soil temperatures at 5 cm depth under bare soil at a nearby field station in Wageningen are presented in Fig. 2.2. In 1993, average daily soil temperatures were already above the minimum germination temperatures of 15 °C or 17 °C early in the season (Fig. 2.2a), while in 1994 (Fig. 2.2b), after storing the seeds and soil at 5 °C during the winter, soil temperatures were lower than in May 1993.

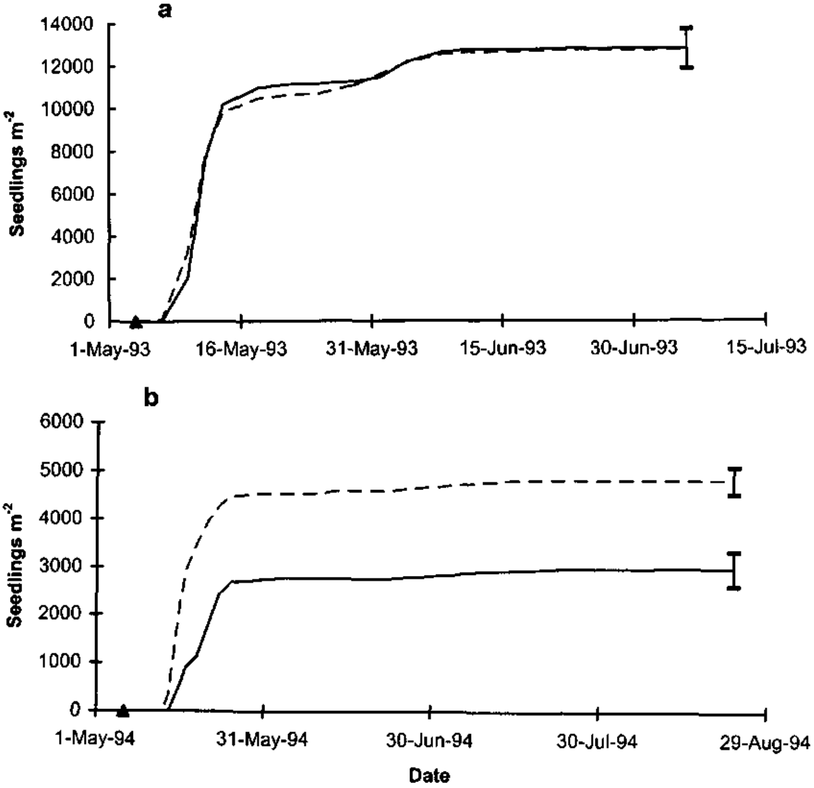


Fig. 2.1 Emergence patterns of susceptible (——) and resistant (– – –) *S. nigrum* from Achterberg in a) 1993 and b) 1994. Vertical bars indicate two times the standard error.

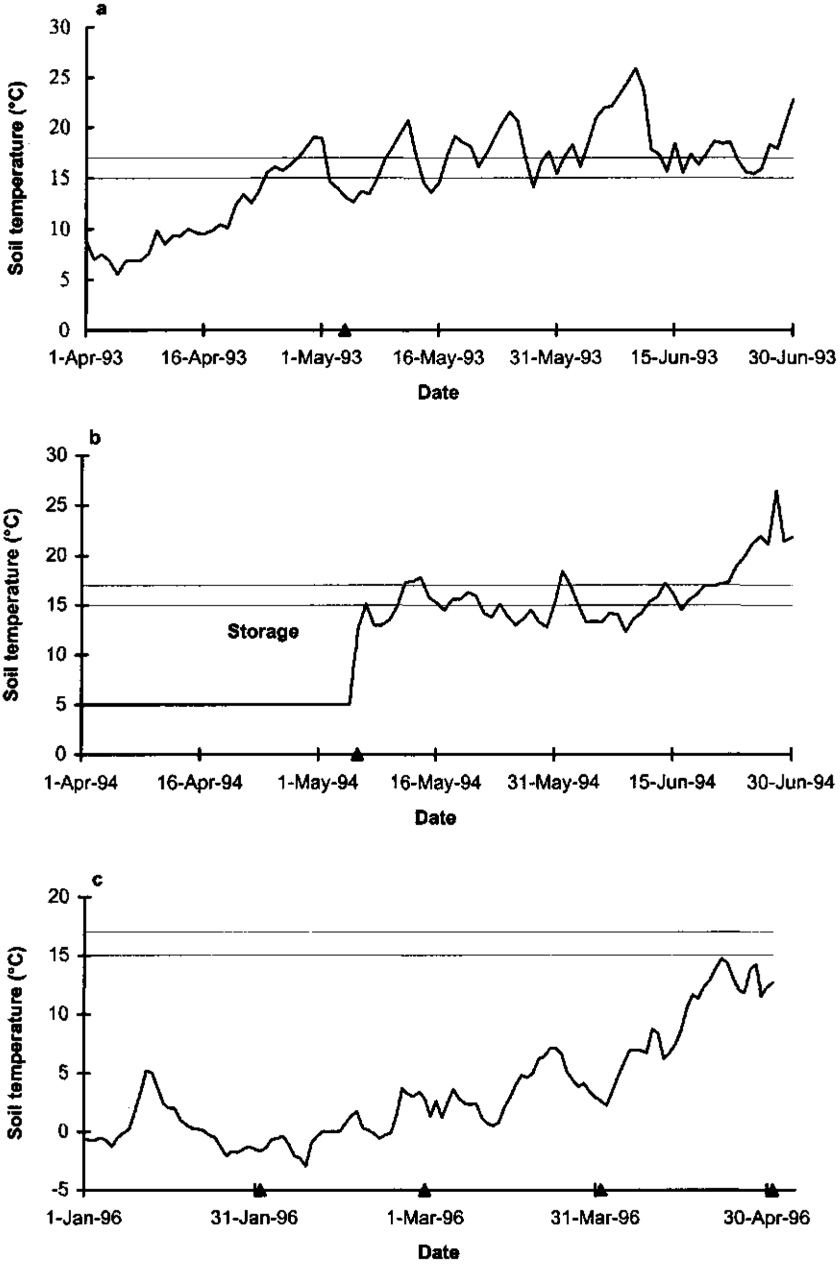


Fig. 2.2 Average daily soil temperatures in 1993 (a), 1994 (b) and in 1996 (c) at 5 cm depth under bare soil. Broken lines indicate minimum temperatures required for germination (Keeley & Thullen 1983; Van den Brand 1985). Triangles in (a) and (b) indicate start of the emergence experiment. Triangles in (c) indicate different times of exhumation.

Germination study

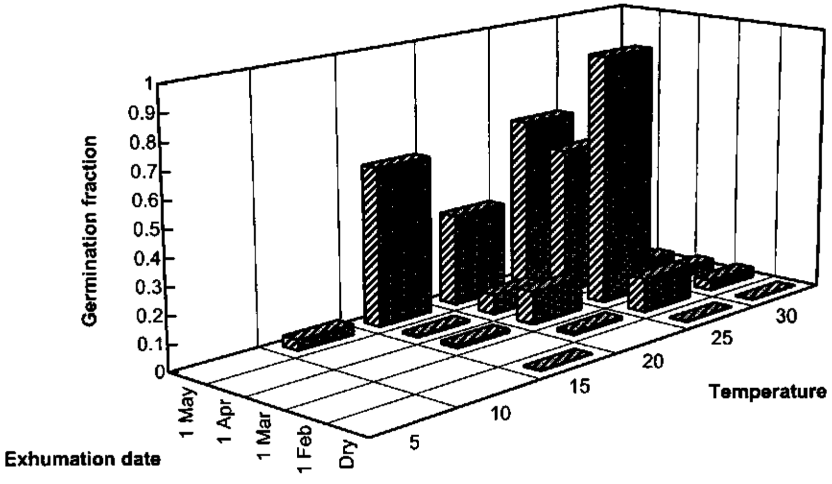
During the germination experiment in 1996, soil temperatures at 5 cm depth were recorded at the same field station in Wageningen (Fig. 2.2c). In Fig. 2.3, the average germination fraction of the three equal sized batches of seed with different seed weights of one biotype are shown. In both populations, biotype had a strong significant effect (Table 2.2) with earlier, faster and greater germination of resistant seeds in all treatments (Fig. 2.3). Minimum temperature requirements for germination of resistant seeds were lower during the whole experimental period. Within biotypes, delaying the time of exhumation generally resulted in an increase in the germination fraction. This increase was population dependent (Table 2.2) and higher for the Achterberg population and especially the susceptible biotype. Germination fraction of dry stored seeds was very low at all temperatures. Seeds exhumed from the field initially showed no germination at 10 °C, but in May exhumations, some germination was observed. Increasing temperatures up to 25 °C increased germination while a temperature of 30 °C inhibited germination. Seed weight had a significant positive effect upon germination (Table 2.2) as is illustrated in Fig. 2.4 for the May exhumation. This effect was independent of exhumation date and biotype. Although significant, the positive effect of seed weight on germination was minimal compared to that of exhumation date and biotype. Data on the germination rate, expressed as $1/t_{50}$ (day^{-1}), is presented in Fig. 2.5, it increased significantly with a delay in exhumation date and as temperature increased within the range used. The highest germination rate was achieved at 30 °C after exhumation on 1 May.

Table 2.2. Results of the stepwise multiple regression analysis. Germination fraction is binomially distributed and germination rate ($1/t_{50}$) is normally distributed. For large numbers of observations the deviance ratio can be interpreted as an F-ratio (Payne *et al.* 1987). Non-significant effects on both parameters are omitted.

	Germination fraction	Germination rate (day^{-1})
Factor	Deviance ratio	F-ratio
Exhumation date	2384.25***	65.44***
Population	68.61***	0.96
Biotype	7230.87***	22.01***
Temperature	4040.17***	294.75***
Seed weight	672.48***	0.03
Exhumation * Population	70.62***	0.30
Exhumation * Biotype	196.18***	2.27
Exhumation * Temperature	131.95***	23.79***
Population * Biotype	885.99***	0.03
Population * Temperature	14.20**	0.34
Biotype * Temperature	30.42***	0.54

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Achterberg, susceptible



Achterberg, resistant

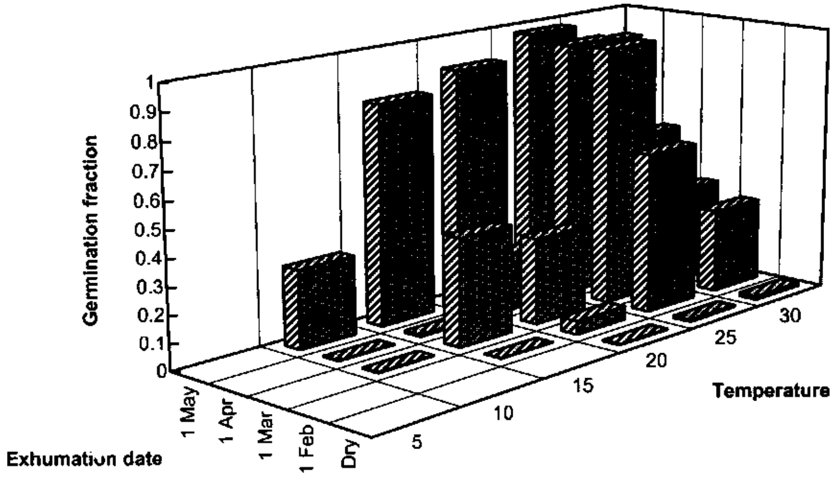
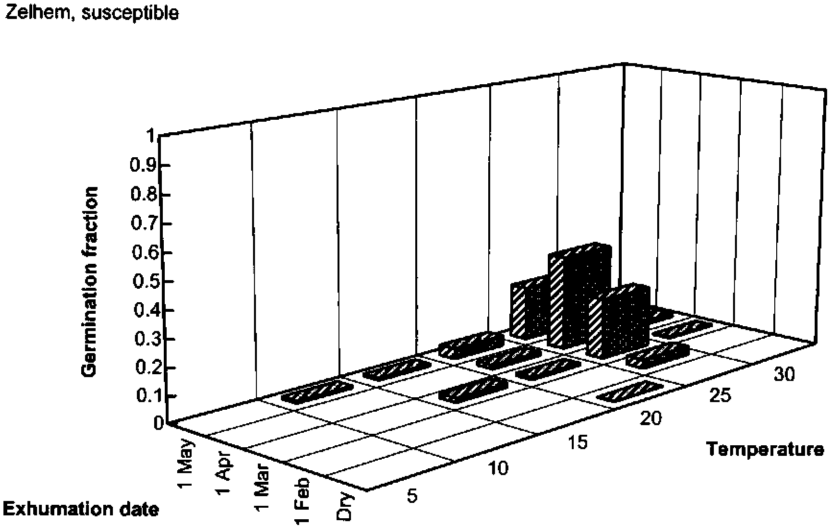


Fig. 2.3(a) Average germination fraction from population Achterberg (a), Zelhem (b) and susceptible and resistant biotypes at different exhumation dates and germination temperatures.


Zelhem, susceptible
Germination fraction
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Exhumation date
1 May
1 Apr
1 Mar
1 Feb
Dry
Temperature
5
10
15
20
25
30

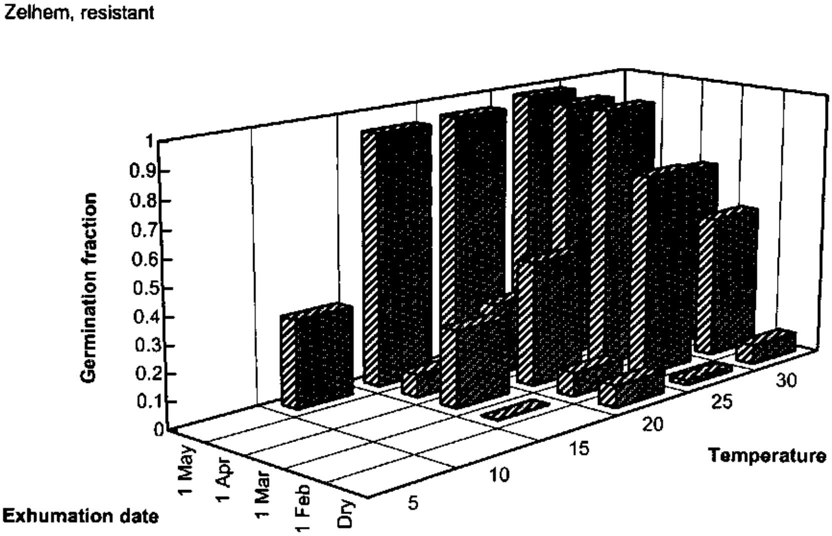

Zelhem, resistant
Germination fraction
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Exhumation date
1 May
1 Apr
1 Mar
1 Feb
Dry
Temperature
5
10
15
20
25
30

Fig. 2.3(b)

Discussion

Emergence patterns of *S. nigrum* have been studied before (Roberts & Lockett 1978, Ogg & Dawson 1984, Van den Brand 1986), but triazine resistant biotypes were not included. In The Netherlands, peak emergence of *S. nigrum* occurred in May (Van den Brand 1986) which agrees with the observed emergence patterns here. Late spring emergence is caused by the high temperature requirements of 17 °C (Keeley & Thullen 1983) or 15 °C (Van den Brand 1985) for germination (Ogg & Dawson 1984).

In the germination experiment, exhumation date had a significant effect on germination. Seed germination increased as seeds were exhumed later in the spring because of rising soil temperatures and subsequent release of dormancy (Bewley & Black 1982). The average daily soil temperature during the exhumation experiment was low (Fig. 2.2c). Exhumation later in the season would probably have resulted in an increased germination fraction of the susceptible biotype because minimum germination temperature requirements would have been met later in the season.

Germination characteristics differed between the two populations. However, when susceptible and resistant biotypes were compared within one population, germination of resistant seeds was much greater than germination of susceptible seeds. Apparently, resistant seeds were less dormant in this particular year. Other reports of germination differences between triazine susceptible and resistant seeds vary because of the different species, geographical origins and germination conditions used. Bulcke *et al.* (1985) found no correlation between dormancy and biotype in *S. nigrum*. In other species, germination characteristics of biotypes have been found to vary but resistant seeds generally have greater dormancy or there has been found to be no correlation between biotype and germination characteristics (Weaver & Thomas 1986, Sereda *et al.* 1996).

Different seed weights of both biotypes from Achterberg gave cause for a separation between effect of seed weight and biotype on germination. The observed positive effect of seed weight on germination fraction of *S. nigrum* agrees with studies on *Rumex* (Cidecyan & Malloch 1982), which showed that larger seeds had a higher germination fraction. However, the positive effect of increased seed weight on germination fraction was relatively small compared to the effect of exhumation date, population and biotype. Germination rate was not affected by seed weight in this experiment. Heavier seeds of other species have been found to have a higher germination rate (Stamp 1990, Hermanutz & Weaver 1991).

The assumed minimum germination temperature of 15 °C of susceptible *S. nigrum* was confirmed by this experiment in incubators with constant temperatures. Once soil temperature in the field had reached 15 °C during part of the day (exhumation date 1 May) there was even germination in the incubator at 10 °C. This suggested that *S. nigrum* has a minimum germination temperature of 15 °C after which a minimum growth temperature is applicable.The minimum temperature for germination of resistant *S. nigrum* was lower than for germination of susceptible *S. nigrum*. Triazine resistant seeds had significantly higher germination at all temperatures and germinated before the soil temperature reached 15 °C.

In 1993, emergence patterns of triazine susceptible and resistant seeds from the Achterberg population were similar but they differed significantly in 1994. Resistant seedlings emerged earlier with a higher emergence rate and the total number of resistant seedlings was significantly higher than the total number of susceptible seedlings.

To explain these different emergence patterns, differences in seed survival can be excluded. Our observations (Chapter 6) show that resistant seeds have a higher mortality, especially during the summer. This finding contrasts with the higher number of resistant seedlings that emerged in 1994 and is, therefore, not an explanation. Different emergence patterns therefore appear best explained by differences in germination characteristics between biotypes.

The lower minimum germination temperature requirement of resistant seeds could explain the varying differences in emergence pattern in the field between 1993 and 1994. In 1993, average soil temperatures exceeded the minimum germination temperature for both biotypes early in the season, resulting in similar emergence patterns. In 1994, soil temperatures were lower and nearly reached the minimum germination temperature of susceptible seeds. This resulted in an earlier, relatively faster and higher fraction emergence of resistant seedlings.

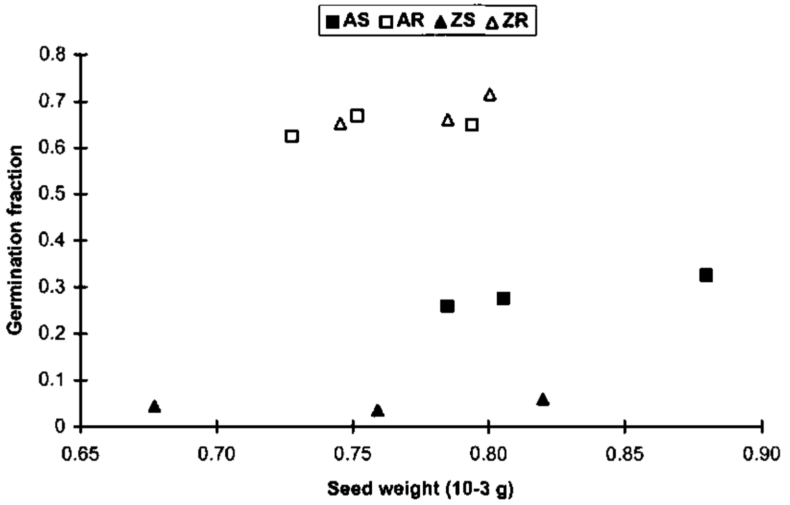


Fig. 2.4 The influence of seed weight on the average germination fraction on 1 May 1996 of the susceptible (AS) and resistant (AR) Achterberg population and the susceptible (ZS) and resistant (ZR) Zelhem population.

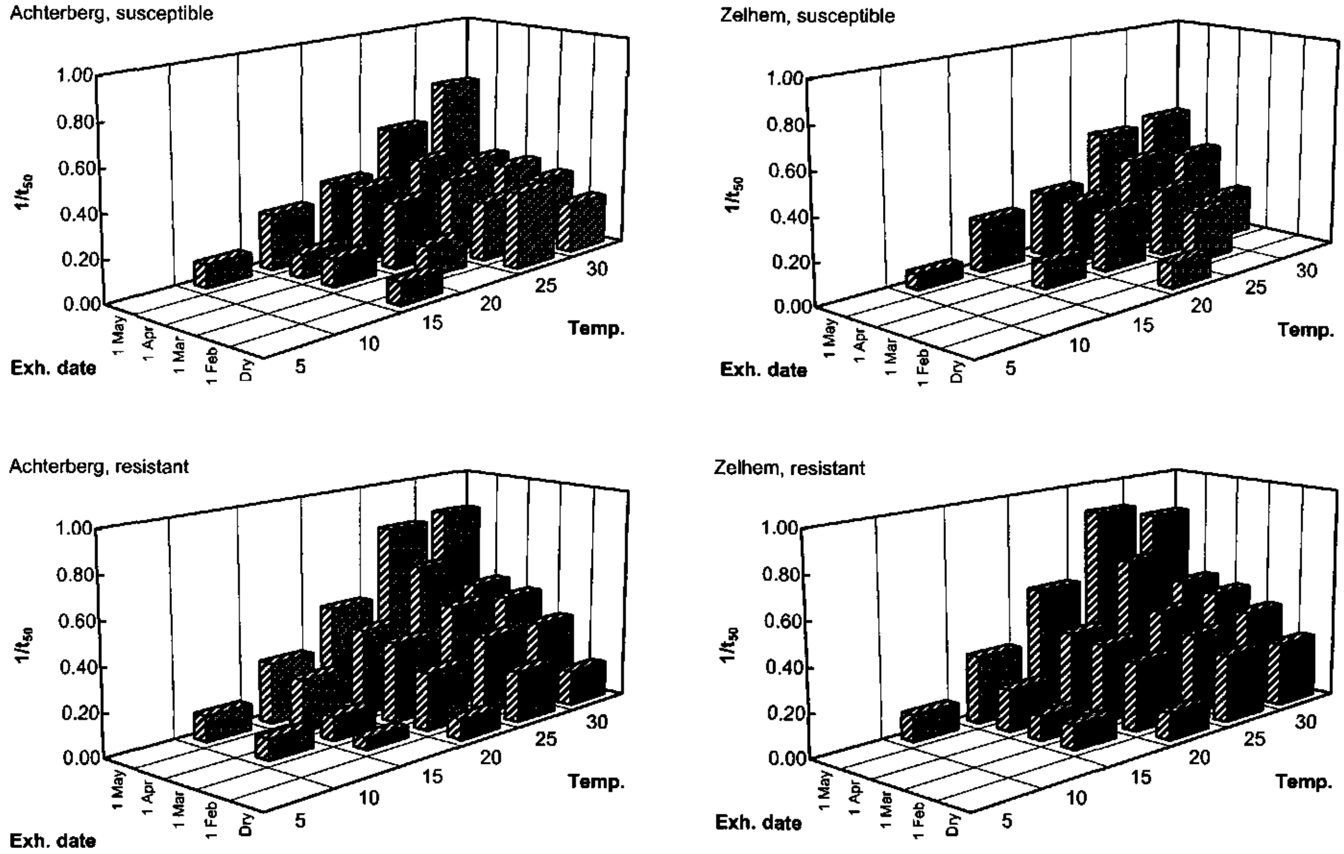


Fig. 2.5. Average germination rate expressed as $1/t_{50}$ (day^{-1}) per population and biotype at different exhumation dates and germination temperatures.

The constant temperatures after exhumation in the germination experiment affected germination as well. In *Sorghum halepense* Benech Arnold *et al.* (1990) showed that fluctuating temperatures released dormancy, and that this effect was determined by the number of cycles, diurnal amplitude, and maximum temperature. Roberts and Lockett (1978) also found in *S. nigrum* a positive effect of high alternating temperatures and the number of diurnal alternations on germination when the upper temperature was above 20 °C. In a cropping system, this influences timing of emergence. In April 1993, soil temperatures alternated above the minimum germination temperature of both biotypes, resulting in no differences in emergence pattern. When the soil and seeds were moved into the field in 1994, soil temperature alternated around the minimum germination temperature of the susceptible biotype and above the minimum germination temperature of the resistant biotype. This probably resulted in a relatively low number of susceptible seedlings.

Management of triazine resistant *S. nigrum* might be possible in some situations using other herbicides. However, the use of these alternative herbicides is not always acceptable. Enhanced knowledge of weed seed ecology may contribute to the development of other weed management options (Forcella *et al.* 1993). Results from our study showed that resistant seeds had a lower minimum germination temperature requirement. This might have implications for triazine resistant *S. nigrum* populations in the field. It diminishes the role of the seed bank as a buffer because resistant seeds will germinate at low temperatures in spring. Within the same temperature range, susceptible seeds will stay dormant and remain in the seed bank. In this way, uncontrolled triazine resistant weed populations may increase very rapidly causing yield loss and, in the case of *S. nigrum* in maize, contamination of the maize fodder crop.

Differences in emergence patterns of both biotypes, depending on soil temperature, could offer extra possibilities to control the number of resistant seedlings. If the triazine resistant biotype emerges earlier it could be controlled by a non-selective herbicide in no tillage or an extra soil cultivation before sowing of the crop. Low soil temperatures combined with an early soil cultivation (stale seedbed preparation) will increase the number of resistant seedlings and subsequent seed bed preparation will kill them. This may decrease the number of triazine resistant plants in the population. Later emerging triazine susceptible *S. nigrum* seedlings can be controlled with many herbicides used in a maize cropping system. However, when soil temperatures in the spring are high, no differences in emergence pattern will be observed and control of resistant seedlings with a stale seed bed preparation or a non selective herbicide will be impossible. To control resistant populations of *S. nigrum* in an integrated weed management system, daily soil temperatures are required.

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Chapter 3

Emergence depth of triazine susceptible and resistant *Solanum nigrum* seeds

Abstract

Effects of population with different origin, biotype, seed size, temperature and depth of burial on the emergence of germinated seeds were investigated to assess the possibilities for successful management of triazine resistant populations of *Solanum nigrum*. Emergence fraction increased with temperature and decreased with increasing depth of placement. Emergence fraction appeared to be highest at intermediate seed weights. Resistant seeds showed a higher emergence fraction than susceptible seeds while emergence rate of resistant seeds was slightly lower. Emergence rate increased with increasing temperatures and decreasing depths. The possibilities for management of resistant *S. nigrum* with a stale seedbed preparation are discussed.

Key-words: black nightshade, triazine resistance, seed size, temperature, resistance management, pre-emergence growth.

Introduction

Triazine resistant *Solanum nigrum* L. is a common weed in silage maize in Europe. Resistant plants survive triazine herbicide applications, compete with the crop, and may contaminate the harvest with poisonous berries. Reduction of the absolute and relative number of resistant plants through crop and weed management is necessary to prevent further spreading and enlargement of resistant weed populations.

Differences between susceptible and resistant biotypes in life history characteristics and fitness have been reported for several weed species in relation to triazine resistance (Holt 1993). To use these differences in life history and fitness for control purposes, insight into the different life cycle processes such as germination and emergence traits, growth characteristics, and seed production of both biotypes is necessary. With this knowledge, integrated weed management strategies of triazine susceptible and resistant *S. nigrum* can be developed.

An important part of successful weed management consists of knowledge of how many seedlings will emerge from which depth and at what time (Mohler 1993, Forcella *et al.* 1993). Weed seeds in the soil seed bank can either stay dormant, germinate and successfully emerge, germinate and die before emergence, decay, or be eaten. Kremer and Lotz (1998, Chapter 2) showed that triazine susceptible and resistant biotypes of *S. nigrum* had different germination characteristics in two populations. Biotypes within each population were collected from the same field. Resistant seeds had lower minimum temperature requirements for germination and may, therefore, germinate earlier in the season. The ultimate number of seedlings is determined by the ability of germinated seeds to emerge successfully from different depths.

After germination at a certain depth, the size of the seed reserve is probably the essential determinant for successful establishment of the seedling (Jurado & Westoby 1992). The deeper the seed is sown, the greater are the resources needed for hypocotyl extension; in general, larger seeds can emerge from greater depths. Part of the variation in seed size is maternally determined. In this chapter, differences in maternal effects because of different growing conditions are excluded as much as possible by growing triazine susceptible and resistant parent plants in a field under similar conditions. Wulff (1986a) stated that seed size is limited either directly or indirectly by resource availability or current photosynthesis. Therefore, the reduced photosynthesis of triazine resistant plants (Holt 1993) could indirectly result in different emergence characteristics of susceptible and resistant seeds. To separate the effect of seed size from that of biotype, seeds of both biotypes were divided in three different weight fractions.

The rising soil temperature in spring also affects emergence characteristics. Emergence fraction and emergence rate increased in flax with increasing temperatures (O'Connor & Gusta 1994) and increased to an optimum temperature in *Malva pusilla* (Blackshaw 1990). Temperatures in our experiment varied from 10 °C to 20 °C, covering the soil temperature range at 5 cm depth in April and May in The Netherlands.

The objective of this study was to examine the effect of biotype and temperature, independent from population and seed weight, on the emergence characteristics of germinated seeds of triazine susceptible and resistant *S. nigrum* from different depths in the soil.

Material and methods

Plant material

In September 1995, ripe berries were harvested from *S. nigrum* plants grown in an experimental field under similar environmental conditions with no inter- or intra-specific competition. Plants originated from populations from two maize fields with both biotypes present in each field; Achterberg (51°59' N, 5°35' E) and Zelhem (51°59' N, 6°18' E). Triazine resistance of plants was confirmed using measurements of quantum yield (Kremer 1995). The collected berries represented 10 to 19 plants per biotype. Seeds were washed, sieved and dried at room temperature. Dry seeds from each biotype and population were divided in three equal-sized batches with different seed weights attained by separation with air, using a South Dakota Seed Blower (model 757, Seedburo Equipment Co., Chicago, USA). Individual seed weights in each batch and their standard errors were determined by weighing 200 single seeds (Table 3.1). Average seed weight of the resistant biotype of the Achterberg population was significantly lower than that of the susceptible biotype. The Zelhem population showed no significant differences in average single seed weights between the two biotypes. In both populations the variation in individual seed weights of the susceptible biotype was larger.

Table 3.1. Individual seed weights of the different fractions of the biotypes from different populations. Standard errors of the means are in parentheses.

Population	Biotype	Fraction	Seed weight (10^{-6} g)
Achterberg	Susceptible	I	784.8 (7.61)
		II	805.4 (6.46)
		III	879.7 (6.71)
	Resistant	I	727.6 (6.71)
		II	751.6 (6.75)
		III	793.9 (6.28)
Zelhem	Susceptible	I	677.3 (8.68)
		II	759.2 (7.96)
		III	820.0 (7.72)
	Resistant	I	745.5 (7.05)
		II	785.1 (6.96)
		III	800.3 (5.97)

The dry stored seeds were placed in petri dishes on moist filter paper to germinate at alternating temperatures of 15/25 °C with 16 h. of light. Germinated seeds with a visible radicle were removed daily and stored at 5 °C from 1-6 days to stop further growth. Root length of the germinated seeds at the start of the experiment never exceeded 3 mm.

Soil characteristics

The study was conducted with a sandy loam soil from an arable field at Wageningen. The soil was collected in February 1996, dried and X-ray radiated with 1 MRad to kill all seeds present. Dry soil was sieved at 5 mm and wetted to a moisture percentage of 14.1 % which corresponded with a pF-value (Korevaar *et al.* 1983) of 2.0. At this pF-value, small soil moisture fluctuations resulted in minor changes in pF.

Emergence experiment

In the emergence experiment, PVC cylinders with a diameter of 5.9 cm and a height of 10 cm were used. The cylinders were filled with soil in different homogeneous layers depending on depth of burial. Soil layers were packed to a fixed penetration resistance of 0.7 MPa, which corresponded with a bulk density of 1.392 g cm^3. Germinated seeds were buried at depths of 1, 2, 4 and 6 cm. At the desired depth of burial, the surface of the soil column was roughened and 20 germinated seeds were homogeneously distributed. The remaining soil layers were put on top of the seeds and compacted in the same way as beneath the seeds. Weight of the filled cylinders was determined to facilitate preservation of the right soil moisture. Evaporation was prevented by covering the cylinder.

Cylinders were placed in 6 incubators with constant temperatures of 10, 15 and 20 °C. Every day, emerged seedlings were counted and removed, and water was added up to the initial weight when necessary. Assessments continued until no emergence was observed for more than three weeks.

Statistical analysis

Experimental set-up consisted of a randomized design with two replicates where all combinations of burial depth, temperature, population, biotype and seed weight were used. Both depth and seed weight were treated as quantitative variables. For each cylinder, final emergence fraction and speed of emergence was determined. Speed of emergence was calculated by fitting a Gompertz curve through the emergence data against time and determine the t_{50} (days to 50% emergence) of the fitted curve. The final emergence fraction had a Binomial distribution and the t_{50} a Normal distribution. Both parameters were analysed with a stepwise multiple linear regression where the explanatory variables were added to the regression model and tested for significance (Payne *et al.* 1987).

Results

The emergence fractions of germinated seeds of the two populations with both biotypes, different seed weights and at different temperatures are shown in Fig. 3.1. Significance levels of effects of the different factors are shown in Table 3.2. The significant effect of biotype was independent from all other factors involved. The resistant biotype showed a significantly higher emergence fraction than the susceptible biotype independent from population, depth, temperature, or seed weight ($P < 0.001$). Average emergence fraction of the resistant biotype was 0.51 compared to 0.41 for the susceptible biotype. There was a significant interaction between depth and every other factor except biotype (Table 3.2). Emergence fraction hardly changed from 1 to 2 cm depth of burial, but strongly decreased from 2 to 4 cm depth (Fig. 3.1). No emergence was observed from a depth of 6 cm in this experiment. Average emergence fractions from 1, 2, 4 and 6 cm depth were respectively 0.91, 0.88, 0.14, and 0. The emergence fraction increased significantly with increasing temperatures showing average values of 0.40 at 10 °C, 0.45 at 15 °C, and 0.52 at 20°C. The population also significantly affected the emergence fraction but no interaction with biotype was observed. Average emergence fraction of seeds from Achterberg was 0.50 compared to 0.42 for seeds from Zelhem.

Median seed weight of resistant seeds was lighter than that of susceptible seeds in Achterberg while median seed weight of resistant and susceptible seeds from Zelhem was similar (Table 3.1). Seed weight had a significant effect on emergence fraction which could be described as a quadratic effect (Table 3.2) that is, the emergence fraction often showed maximum values at the intermediate seed weights within populations and biotypes (Fig. 3.1). The maximum emergence fractions at intermediate seed weights depended on temperature and depth.

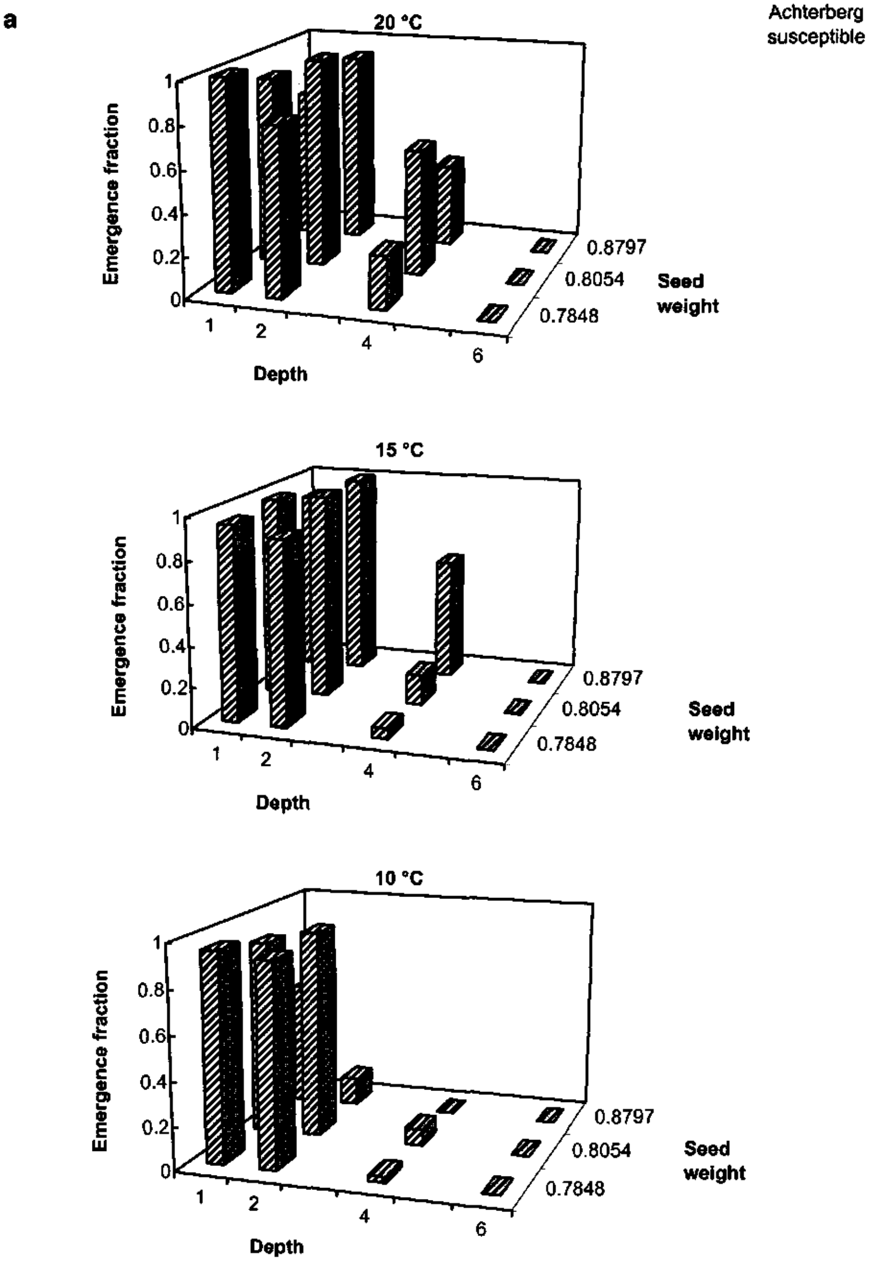
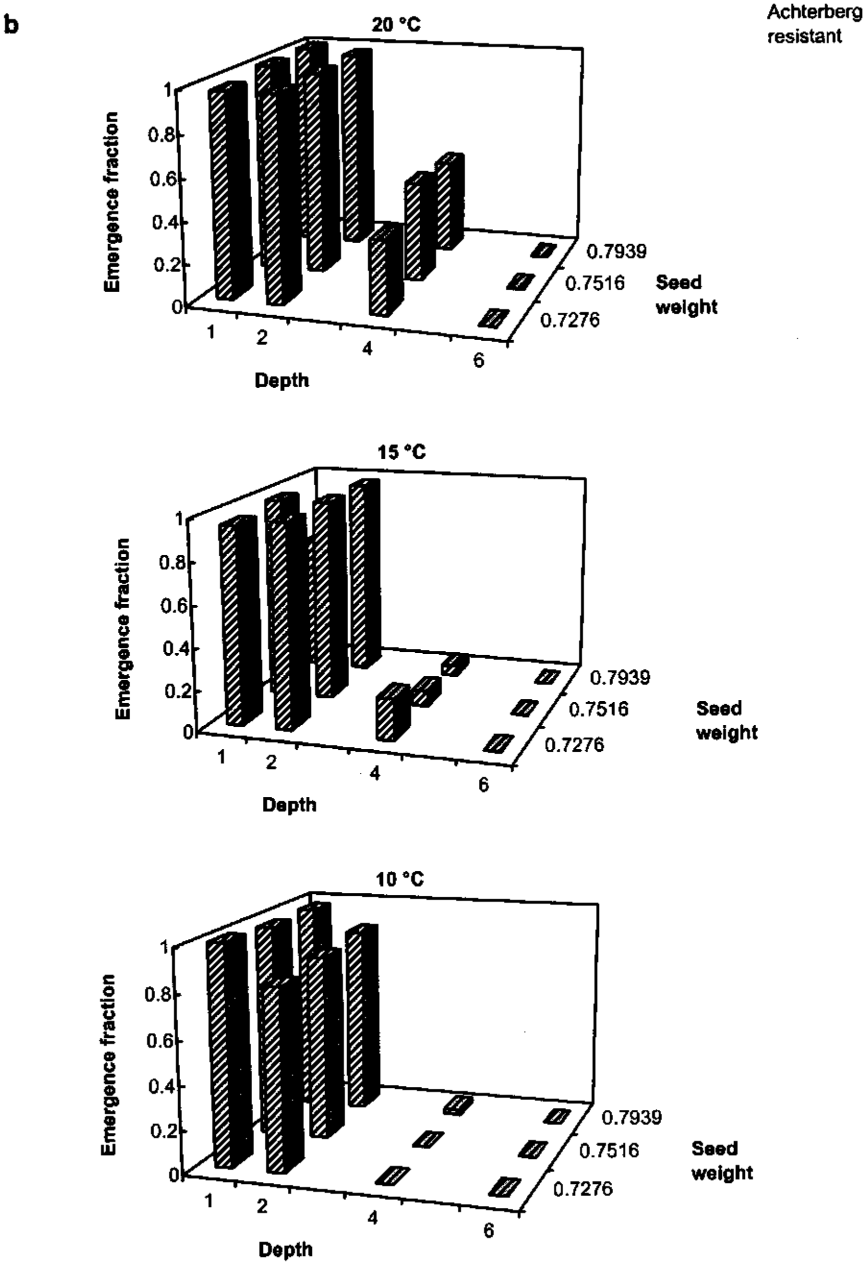


Fig. 3.1 Emergence fraction of germinated seeds with different weights (10^{-3} g) from different depths (cm) at different temperatures. Represented are the triazine susceptible (a) and resistant (b) Achterberg population and the triazine susceptible (c) and resistant (d) Zelhem population.



20 °C

15 °C

10 °C

Fig. 3.1 (b)

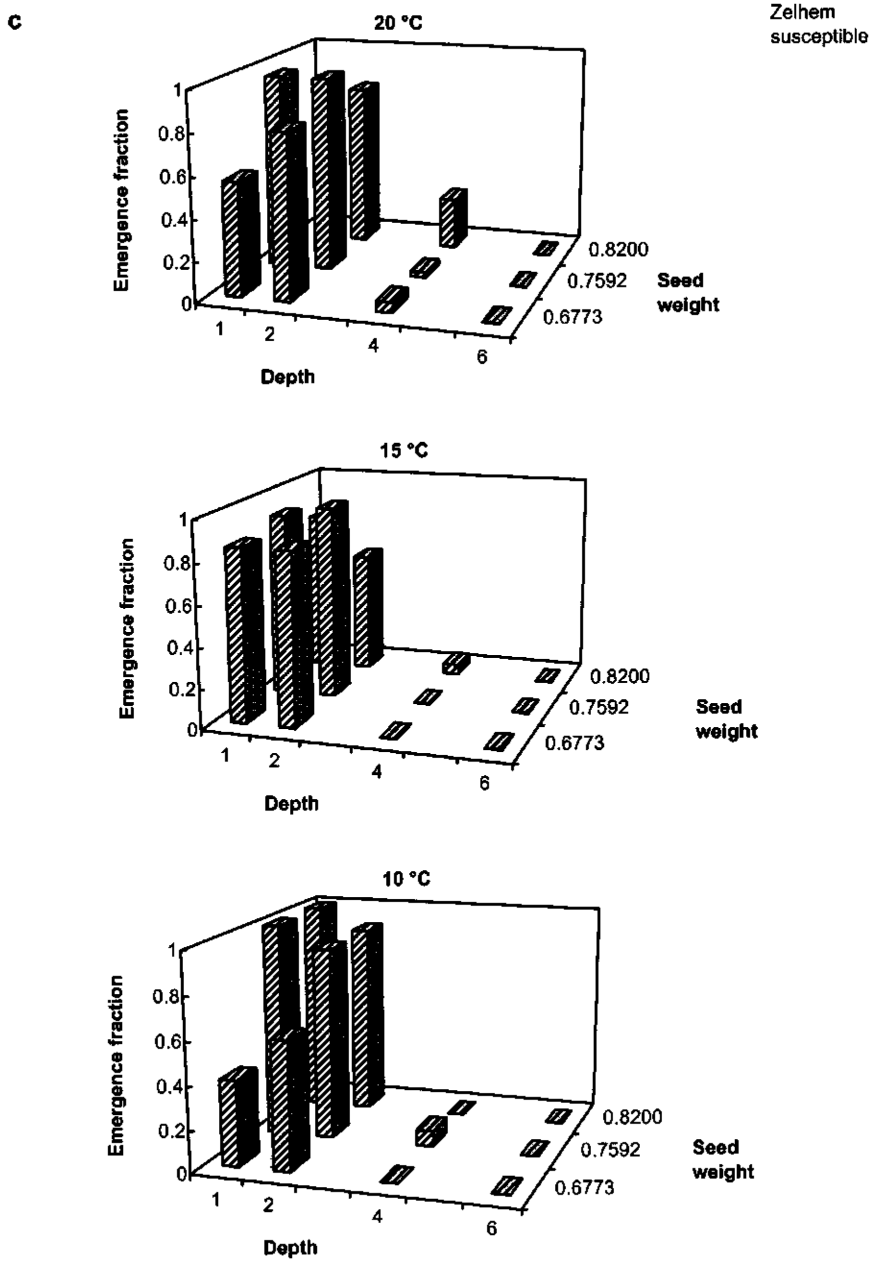

c
Zelhem
susceptible
20 °C
Emergence fraction
0
0.2
0.4
0.6
0.8
1
Depth
1
2
4
6
Seed weight
0.6773
0.7592
0.8200
15 °C
Emergence fraction
0
0.2
0.4
0.6
0.8
1
Depth
1
2
4
6
Seed weight
0.6773
0.7592
0.8200
10 °C
Emergence fraction
0
0.2
0.4
0.6
0.8
1
Depth
1
2
4
6
Seed weight
0.6773
0.7592
0.8200

Fig. 3.1 (c)

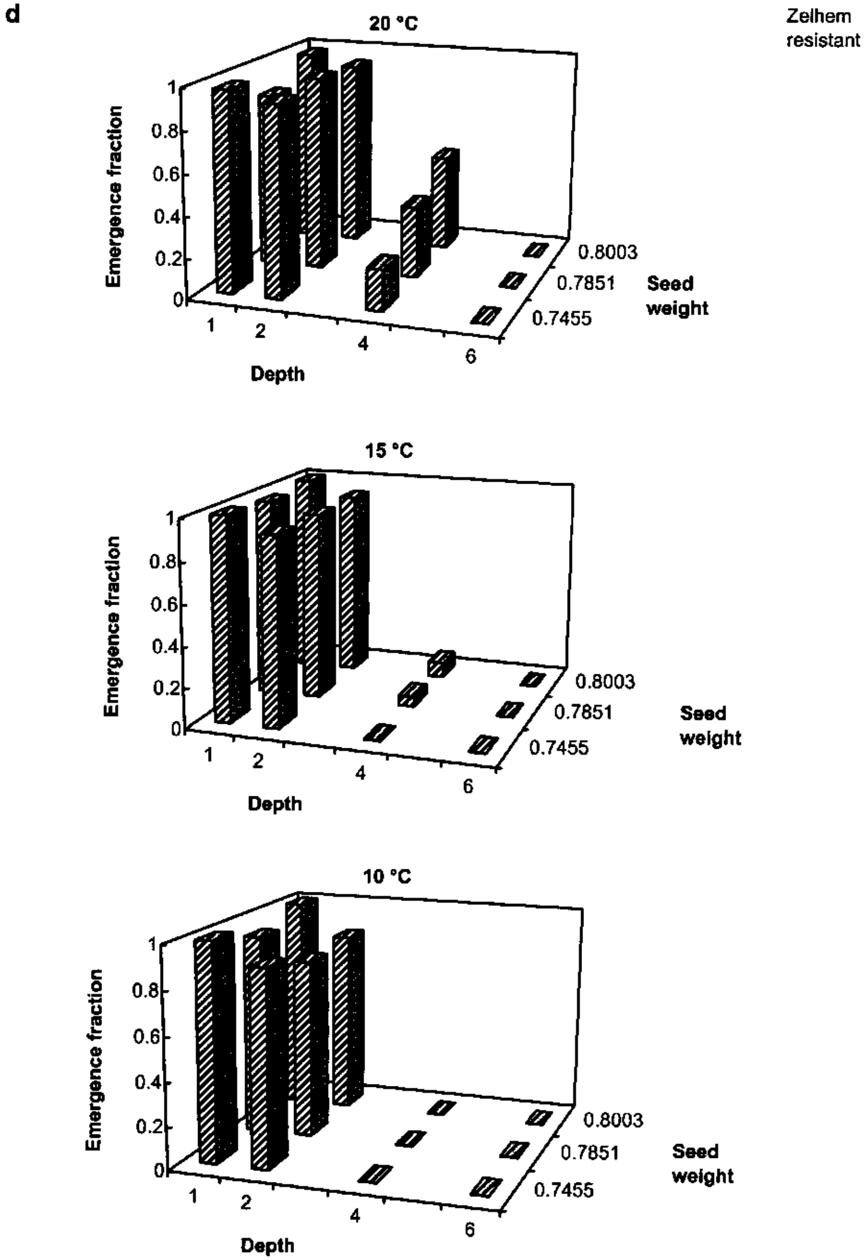

d
Zelhem
resistant
20 °C
15 °C
10 °C
Emergence fraction
0
0.2
0.4
0.6
0.8
1
Depth
1
2
4
6
Seed
weight
0.7455
0.7851
0.8003

Fig. 3.1 (d)

Rate of emergence, expressed as t_{50} (days to 50% emergence), was only affected by biotype, temperature, depth, and their interactions (Table 3.2). The time to 50% emergence increased with increasing depths and decreasing temperatures (Fig. 3.2). Since emergence rate from 4 cm at 10 °C was based on averages from only three observations for the susceptible biotype and one observation for the resistant biotype it was not presented. Resistant seedlings emerged slower than susceptible seedlings. The t_{50} from susceptible seedlings was between 3% (4 cm depth, 20 °C) and 21% (2 cm depth 10 °C) lower than that of resistant seedlings. Population had no significant effect on speed of emergence.

Discussion

The depth from which germinated seeds can emerge in annual species determines which part of the seed bank may produce seedlings. Successful emergence from greater depths will result in higher numbers of emerged seedlings. If all emerged seedlings can subsequently be controlled, the population will be further reduced. Integrated weed management strategies depend on knowledge of timing and number of emerged seedlings (Forcella *et al.* 1993).

Elimination of the effect of different seed weights between biotypes and populations in this experiment enabled us to analyse the actual effect of biotype and population on

Table 3.2. Results of the stepwise multiple linear regression. Significance levels of the different factors on emergence fraction and time to 50% emergence (t_{50}). Germination fraction is binomially distributed and t_{50} is normally distributed. For large numbers of observations the deviance ratio can be interpreted as an *F*-ratio (Payne *et al.* 1987). Non-significant effects on both emergence fraction and t_{50} are omitted.

Factor	Emergence fraction Deviance ratio	t_{50} *F*-ratio
Temperature	8.15*	52.23***
Depth	1026.21***	485.47***
$(\text{Depth})^2$	38.83***	ns
Population	7.00**	ns
Biotype	28.22***	19.23***
$(\text{Seed weight})^2$	35.13***	ns
Depth×Temperature	20.00***	51.66***
Depth×Population	5.16*	ns
Depth×Biotype	ns	4.74**
Depth×Seed weight	9.67**	ns
Depth×$(\text{Seed weight})^2$	5.77*	ns
Temperature×Biotype	ns	4.43*
Temperature×$(\text{Seed weight})^2$	9.28***	ns
Seed weight×$(\text{Depth})^2$	6.98**	ns

ns not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

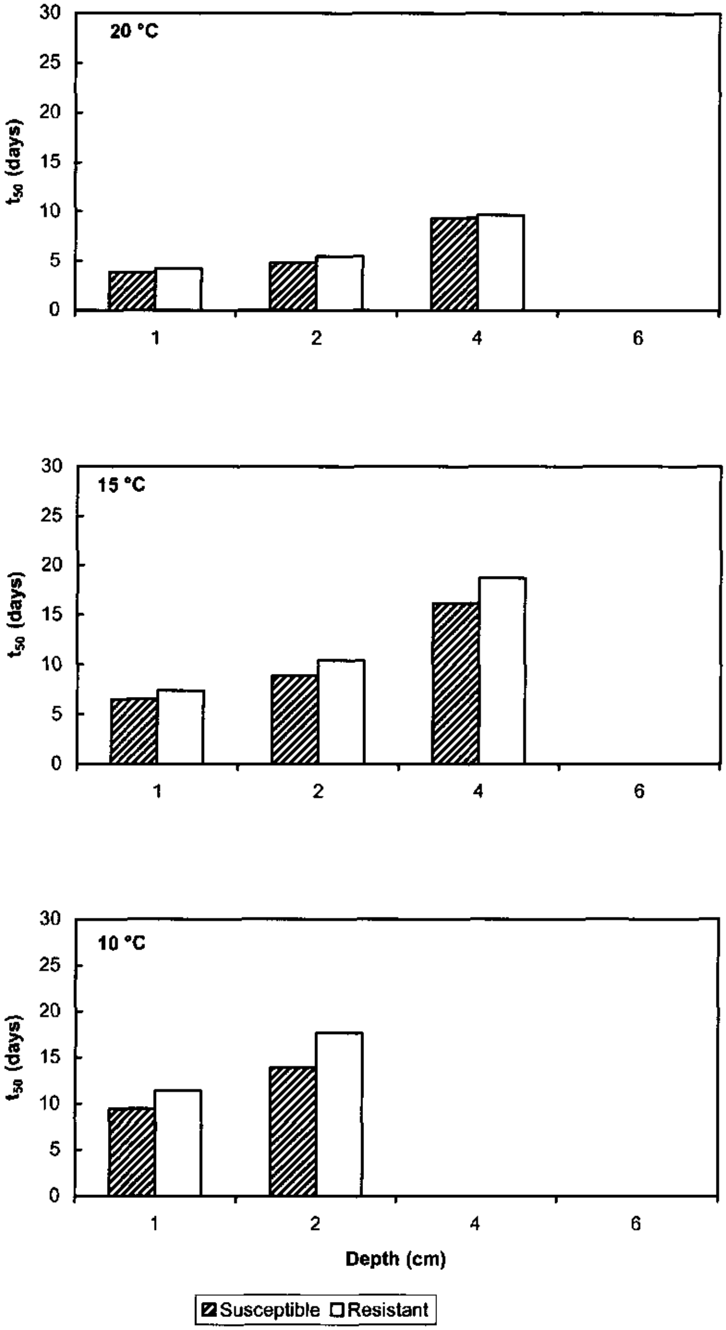


Fig. 3.2 Time to 50% emergence (t_{50}) in days from susceptible and resistant seeds depending on temperature and burial depth.

the emergence fraction. The effect of biotype on emergence fraction showed a slightly higher emergence fraction of resistant seeds. This effect was independent from depth, temperature, seed weight and population. Since no interaction with population was observed, the importance of genomic control and the effect of differential selection on the nuclear genome seems to be limited. The higher emergence fraction of the resistant biotype might, therefore, be a secondary effect of the triazine resistance mutation like in *Poa annua,* where Touraud and colleagues (1987) found a relatively lower abscisic acid content in the triazine resistant seeds. One of the functions of abscisic acid is inhibition of shoot growth and elongation by counteracting gibberellic acid (Brock & Kaufman 1991). Differences in emergence between biotypes were consistent over the two populations. The effect of population on emergence fraction was significant, but depended on depth (Table 3.2).

In general, the emergence fraction decreased with increasing depths. However, in some cases the emergence fraction at 1 cm depth was lower than that at 2 cm depth which seems counter intuitive. This might have been an artefact of the experimental set-up. The penetration resistance in the 1 cm thick soil layer was probably less homogeneously distributed than in the thicker soil layers resulting in relatively more damaged roots and reduced emergence fractions.

Maximum emergence depth of seeds depends on the amount of reserve substances and, therefore, seed weight. Froud-Williams *et al.* (1984) reported for most arable weed species with a seed weight range which included that of *S. nigrum* a critical emergence depth of 5 cm. In this experiment, *S. nigrum* emerged from 4 cm depth while no emergence from 6 cm was observed. The relatively high penetration resistance (0.7 MPa) in the experiment compared to arable field conditions probably prevented *S. nigrum* seeds from emerging from greater depths. Vleeshouwers (1997) showed a strong negative effect of soil penetration resistance on emergence of *Polygonum persicaria*, *Chenopodium album* and *Spergula arvensis* within a range of 0.4 - 1.0 MPa. Earlier reported effects of soil aggregate size on emergence (Cussans *et al.* 1996) were avoided using sandy loam soil sieved at 5 mm.

Within species, most of the physiological responses are proportional to seed size and seed size variability may have a large impact on seedling establishment and survival (Wulff 1986a,b). For example, in *Xanthium strumarium*, seedlings from larger sized seeds had a larger leaf area and a bigger primary root length (Zimmerman & Weis 1983). The effect of seed weight, independent from population and biotype, on emergence fraction of *S. nigrum* was significantly quadratic (Table 3.2), showing maximum emergence at intermediate seed weights. In general, the emergence fraction increases with increasing seed weight (Jurado & Westoby 1992) but a quadratic effect of seed size on emergence fraction was reported for the emergence of *Panicum virgatum* in a sandy soil (Aiken & Springer 1995). However, no possible explanations were mentioned. One hypothesis could be that increasing seed weights have a positive effect on emergence fraction to an optimum but that a trade off with cotyledon size is involved in compacted soils, resulting in a reduced emergence fraction of heavier seeds with larger cotyledons. This hypothesis should be tested in experiments with different soil compactions and soil structures.

The emergence fraction of the resistant seedlings at all depths was higher than that of the susceptible seedlings while the emergence rate of the resistant seedlings was lower. Emergence rate of triazine susceptible and resistant *S. nigrum* was not affected by seed weight while in other species a positive effect of seed weight on emergence rate was observed (Wulff 1986b, Harper & Obeid 1967).

Knowledge of emergence characteristics is required to develop integrated management strategies of populations with triazine susceptible and resistant *S. nigrum*. The depth from which susceptible and resistant seeds can emerge after germination determines how many seedlings of both biotypes will emerge in the field. Previous research showed a lower minimum germination temperature requirement for the resistant seeds compared to that of the susceptible seeds resulting in an earlier germination of triazine resistant seeds in the season (Kremer & Lotz 1998, Chapter 2). This difference in time of germination may offer possibilities to specifically control resistant seedlings before crop emergence i.e. with a stale seed bed preparation or a non-selective herbicide in no-tillage. An important condition for the success of this weed control method is a high number of resistant seedlings with a high emergence rate. This chapter showed an increased emergence fraction of the resistant seedlings from different depths resulting in a higher number of resistant seedlings and a slightly lower emergence rate of the resistant biotype compared to that of the susceptible biotype. This difference decreased with increasing temperature and was small compared to the differences in germination time in spring. The employment of a stale seedbed preparation to reduce triazine resistance levels in the field looks, therefore, promising, but more research is needed to verify this under field conditions.

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Chapter 4

Comparative growth of triazine susceptible and resistant biotypes of *Solanum nigrum* at different light levels

Abstract

The effect of light intensity on growth of plants from five different populations of different origin of triazine susceptible and resistant *Solanum nigrum* was studied in growth chambers at three light levels. Plants were grown without competition and with optimal mineral nutrition. After 29 days, biomass of the resistant biotypes was about 25% less than that of the susceptible biotypes at every light level. Classical growth analysis showed that this was the result of a lower initial biomass of the resistant biotype at the start of the experiment as the relative growth rates (RGR) of the susceptible and the resistant biotype in the early growth phase were equal. The most important growth component accounting for the RGR is the Specific Leaf Area which was higher for the resistant biotype which was compensated by a lower Net Assimilation Rate (NAR). The fraction dry matter invested in leaves was equal for both biotypes but the resistant biotype produced more leaf area per unit leaf weight. The equal RGR of the susceptible and resistant biotype in the early growth phase may have implications for the competitive ability and the population dynamics of a population with resistant biotypes.

Key-words: black nightshade, growth analysis, light level, management strategies, RGR, SLA, triazine resistance, weed control.

Introduction

The occurrence of herbicide resistant weeds is a major concern in different cropping systems around the world. In maize, the intensive use of triazine herbicides for several decades has resulted in triazine resistance in several weed species. Examples in The Netherlands are *Chenopodium album* L. and *Solanum nigrum* L. (Van Oorschot & Straathof 1988). Plants resistant to triazine herbicides might also be resistant to other herbicides, seriously limiting the options for chemical control when the number of available herbicides decreases as well. Therefore, ecologically based management strategies for triazine resistant weeds should be developed. To achieve this, more knowledge about the life history and ecology of triazine susceptible and resistant biotypes of *S. nigrum* is required.

An important part of the life history of a weed is its establishment under a crop canopy. Successful establishment is mainly determined by the duration of the period between crop and weed emergence (Kropff & Van Laar 1993). Plant growth early in the season plays a crucial role in determining the size of the plants when competition starts in arable fields (Sattin & Sartorato 1997). Final plant weight and reproduction are often related to early season growth rate. When plants grow exponentially, the relative growth rate (RGR) is a stable and useful parameter for comparison of growth between species. The definition of RGR is independent of size of plants and represents the rate of increase in plant weight per unit plant weight already present. RGR is often divided into the components NAR (g (plant) m^{-2}(leaf) d^{-1}), LWR (g (leaf) g^{-1}(plant)) and SLA (m^2(leaf) g^{-1}(leaf)) to unravel causes of variation in growth rate: $RGR = NAR \times LWR \times SLA$ (Hunt & Cornelissen 1997).

Different growth characteristics of triazine susceptible and resistant weeds have been compared and interpreted in terms of a reduced fitness of the resistant biotype in the absence of the herbicide. Triazine resistance results from a mutation in the chloroplast genome, reducing the electron transport rate between Q_A and Q_B in photosystem II. As a result, lower photosynthetic rates, biomass production, fecundity and competitiveness of resistant biotypes have been found in several experiments reviewed by Holt *et al.* (1993). However, in other experiments, susceptible and resistant biotypes showed an equal biomass production in the absence of competition (Schönfeld *et al.* 1987, Anderson *et al.* 1996). This could be explained by the maternal inheritance of the triazine resistance, resulting in a possible compensating effect of genome controlled traits on the reduced biomass production of the resistant biotype (McCloskey & Holt 1990). Another explanation might have been the light levels used in the experiments, since resistant biotypes are more sensitive to photo-inhibition at a high light level (PFD = 1200 $\mu mol\ m^{-2}\ s^{-1}$) while under low light (PFD = 100 $\mu mol\ m^{-2}\ s^{-1}$) differences in growth between biotypes are much less clear (Hart *et al.* 1992).

The effect of biotype on growth may be confounded with possible compensating effects of genome controlled traits. The nuclear effect should be eliminated by the use of nuclear isogenic biotypes or several different populations with both biotypes present (Warwick & Black 1993). The required number of populations depends on the size of the real difference between biotypes and the variability both between and within populations (Cousens *et al.* 1997).

The precise effect of light level on weed growth, as perceived under a closing canopy, is best determined under homogeneous environmental conditions in a growth chamber. Variable weather conditions as they occur in the field do not play a role, and growth of both biotypes can be compared under low light, but resource-rich, undisturbed conditions with no competition between individual plants.

The objective of this study was to determine the effect of biotype and population on growth and development of young *S. nigrum* plants under different light levels and to identify the growth parameters responsible for possible differences.

Material and methods

Triazine susceptible and resistant *S. nigrum* plants were collected in 1995. In five different fields (Table 4.1), representing five populations, both susceptible and resistant seedlings were collected, identified by measuring chlorophyll fluorescence, and were grown in the glasshouse under similar environmental conditions. Ripe berries were collected and seeds were washed, sieved and dried at room temperature. Average

Table 4.1. Geographical locations of the different fields and average single seed weight (10^{-3} g) of the triazine susceptible and resistant biotypes of *S. nigrum*.

Population origin	Geographical location	Seed weight (mg)	
		Susceptible	Resistant
Achterberg	51°59'N, 5°35'E	0.8200	0.7518
Zelhem	51°59'N, 6°18'E	0.7615	0.7700
Gorssel	52°12'N, 6°11'E	0.8715	0.6980
Epse	52°13'N, 6°12'E	0.8563	0.7707
Beerze	52°31'N, 6°32'E	0.8720	0.8278

single seed weight was determined by weighing 600 seeds. Germination characteristics of the different populations and biotypes were determined in a germination experiment with alternating temperatures of 15/25 °C and 12 h of light. Final fraction germination and time to 50% germination (t_{50}) were determined (Table 4.2).

To ensure sufficient germination in the growth experiment, seeds were stored at 5 °C in a 1 ppm gibberellic acid solution for two weeks prior to the start of the experiment to break primary dormancy. Subsequently, the seeds were thoroughly rinsed with tap water after which they were laid out in petri dishes on moist filter paper to germinate at alternating temperatures of 15/25 °C with 12 h of light. Germinated seeds with a visible radicle were removed daily and stored in darkness at 5 °C from 1-6 days to stop further growth. Root length of the germinated seeds used in the experiment never exceeded 3 mm.

At the start of the experiment, the germinated seeds were placed in rock wool floating on an aerated nutrient solution. Nutrient concentrations were: 1.0 mM NH_4, 6.5 mM K, 2.75 mM Ca, 1.0 mM Mg, 10.75 mM NO_3, 1.5 mM SO_4, 1.25 mM P, 15 μM Fe, 10 μM Mn, 4 μM Zn, 20 μM B, 0.75 μM Cu and 0.5 μM Mo. The Photosynthetic Active Radiation (PAR) level at the rock wool surface valued 500 ± 2 $\mu mol\ m^{-2}\ s^{-1}$ with a day/night temperature regime of 19/15 °C. After 13 days, the seedlings had two true leaves and were transferred to the growth chambers with different light treatments. The experimental design consisted of a split-plot design with

Table 4.2.Average final germination fraction and t_{50} of seeds from different populations and biotypes. Asterisks indicate significant differences between resistant and susceptible biotype based on a *t*-test.

Population	Final fraction germination		t_{50} (days)	
	Susceptible	Resistant	Susceptible	Resistant
Achterberg	0.99	0.99	4.5	3.6**
Zelhem	0.59	0.98***	6.0	3.1***
Gorssel	0.21	0.99***	5.8	3.9***
Epse	0.05	0.99***	6.5	3.8**
Beerze	0.09	1.00***	6.5	4.5*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

three replicates (different chambers) with light level treatments on main plots. Population, biotype and harvest time were randomized within subplots. Each replicate consisted of one growth chamber which was divided into three compartments by curtains which already slightly reduced the light levels in the compartments. Light in each compartment was provided by 400 W HPI lamps and 400 W AGRO SON lamps in a 1:1 ratio supplemented with four 40 W light bulbs and one fluorescent tube. Within the compartments, layers of cheese cloth were used to further reduce the light to different levels in the treatments without change of the spectrum which was checked by light spectrum measurements in each compartment. Average photosynthetic photon flux densities (PPFD) at the rock wool surface were 376.5 (±2.3), 212.5 (±1.8) and 132.5 (± 2.1) μmol m^{-2} s^{-1}, representing 100%, 56% and 35% light respectively. The 100% light level before and after transfer of the plants differed because of the curtains which reduced light levels. Climate in the growth chambers was maintained at a 14 h daylength with a day/night temperature of 18/12 °C and a minimum relative air humidity of 70%.

Single plants from each treatment were harvested in three replicates at 0, 6, 11, 15, 20, 25, and 29 days after transfer to the different light levels. At each harvest, height of the plant, leaf area, dry matter of leaves, stems, and roots and numbers of forks, flowers and branches of every single plant were determined. The obtained data were analysed by ANOVA based on a split-plot design (Payne *et al.* 1987).

Growth was analysed by fitting an exponential-linear growth equation because plants did show the initiation of linear growth as a result of mutual shading of leaves (Goudriaan & Monteith 1990):

$$W = (c_m/r_m) \times \ln(1 + \exp(r_m \times (t-t_b))) \qquad \text{(Eq. 4.1)}$$

In this equation, the total plant dry weight (W) is determined by the initial maximum relative growth rate (r_m) and the maximum absolute growth rate (c_m). The parameter t represents time and t_b is the time where growth passes from exponential growth to linear growth. The goodness of fit was determined by checking the percentage variance accounted for and the residuals of the fitted values (Payne *et al.* 1987). The relative growth rate (RGR) in the early growth phase was used in the growth analysis.

RGR can be expressed in two components: the Net Assimilation Rate (NAR; g(plant) m^{-2} d^{-1}) and Leaf Area Ratio (LAR; m^2 g^{-1}(plant)):

RGR = NAR×LAR.

The NAR was derived from data from adjacent harvest intervals. The LAR was further subdivided in Leaf Weight Ratio (LWR; g (leaf) g^{-1}(plant)) and Specific Leaf Area (SLA; m^2 g^{-1}(leaf)):

LAR = LWR×SLA

The LWR and SLA were derived for data obtained at each single harvest. The parameters RGR, NAR, LAR, SLA and LWR were analysed by ANOVA based on the split-plot design of the experiment.

Results

In most populations, the single seed weights of the resistant biotype were lower than those of the susceptible biotype (Table 4.1). The final germination fraction and germination rate of the susceptible biotype were generally much lower than that of the resistant biotype (Table 4.2). Initial dry weight of the seedlings at the start of the experiment increased slightly with seed weight ($P < 0.05$) but was mainly determined by biotype ($P < 0.001$). Initial dry weights of the resistant seedlings varied between 33% and 67% of the weight of susceptible seedlings (Table 4.3). The resistant seedlings had a clear delay in biomass production at the start of the experiment.

Total dry matter production at the end of the experiment was higher for the susceptible biotype ($P < 0.001$) and increased with light level ($P < 0.01$; Fig. 4.1). The factors biotype and light interacted significantly ($P < 0.05$). At the highest light level, the total dry matter production of the susceptible biotype was 25 % higher than that of the resistant biotype. Origin of population had no effect on the total dry matter production of susceptible and resistant biotypes. The final leaf area per plant at the highest light level totalled 3501 and 2793 cm^2 for the susceptible and resistant biotype, respectively.

The accumulation of dry matter in time, measured in subsequent harvests of single plants, is best analysed by curve fitting, making the final results less erratic. Fitting an expolinear growth equation resulted in a good fit based on percentage variance accounted for and the residuals of the fitted values. Comparisons of the fitted RGR with the RGR calculated from adjacent harvest intervals showed the high variation in RGR's based on harvest intervals (Fig. 4.2). The homogeneous distribution justifies the use of the fitted RGR in the growth analysis. Relative growth rates of the resistant biotypes were not different from those of the susceptible biotypes (Fig. 4.3). Relative growth rate increased with increasing light level ($P < 0.001$). No effect of population or biotype on the RGR was observed. Values of c_m and t_b from the fitted expolinear growth equation are presented in Table 4.4. The maximum linear growth rate (c_m) tended to be affected by the interaction of light×biotype ($P < 0.10$). The time at which the growth rate passes from exponential to linear (t_b) decreased with increasing light

Table 4.3. Average total dry weight (10^{-3} g) of seedlings at the start of the experiment (day 0; n=3). Standard errors in brackets. Asterisks indicate significant differences between resistant and susceptible biotype based on a *t*-test.

Population	Biotype	
	Susceptible	Resistant
Achterberg	8.9 (0.70)	6.0 (0.62) *
Zelhem	10.0 (3.25)	5.7 (1.24)
Gorssel	15.3 (2.92)	5.1 (0.62) *
Epse	12.7 (0.79)	6.8 (1.27) ***
Beerze	10.2 (0.72)	6.2 (1.19) *

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

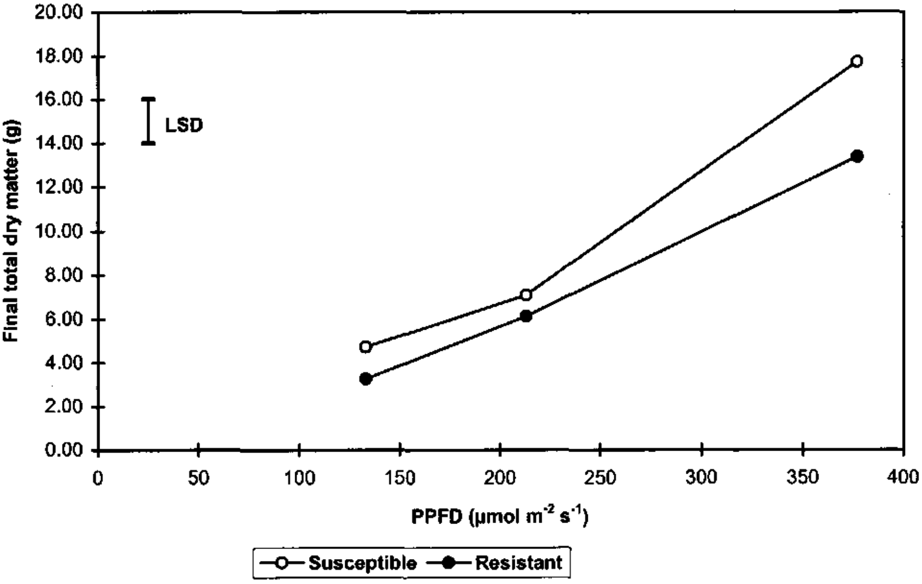

Fig. 4.1 Total dry matter of the triazine susceptible and resistant biotype in relation to light intensity after 29 days. Error bar indicates Least Significant Difference.

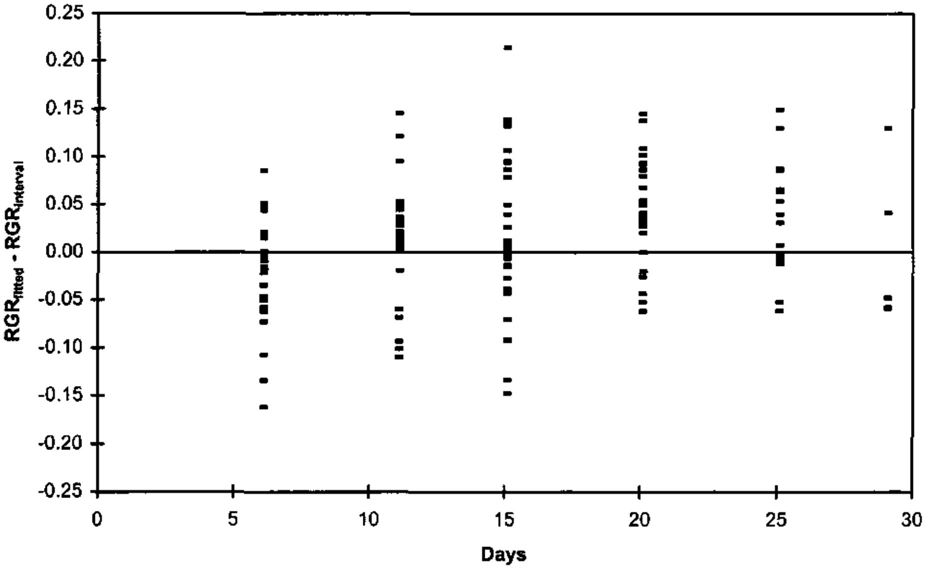

Fig. 4.2 Differences between the RGR fitted with the expolinear equation up to the linear phase and the RGR based on growth between adjacent harvest intervals.

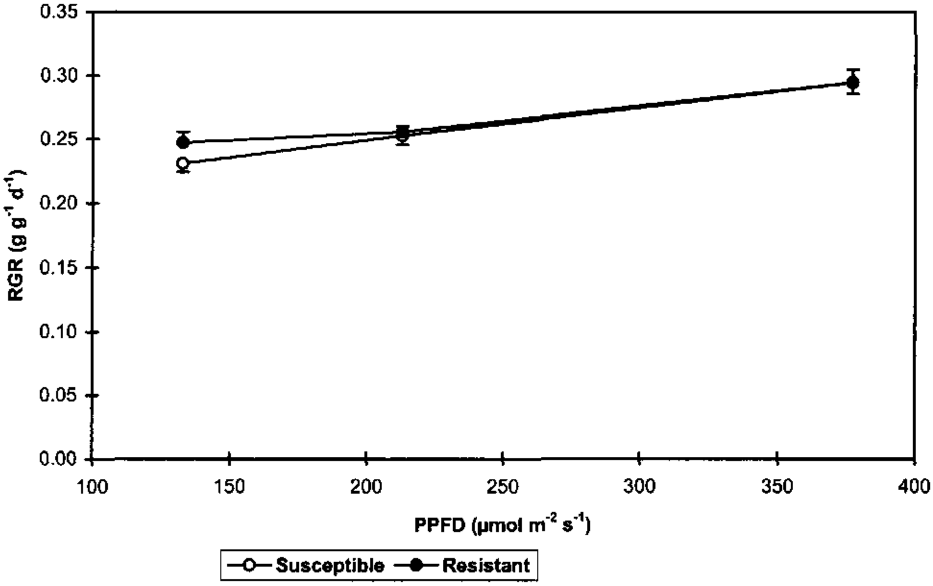

Fig. 4.3 Relative Growth Rate (total dm. total dm.$^{-1}$ d^{-1})of triazine susceptible and resistant *S. nigrum* plants grown at different light levels. Error bars indicate the standard error.

levels ($P < 0.01$) and also tended to be affected by the interaction of light×biotype ($P < 0.10$).

The RGR component NAR (g m^{-2} d^{-1}), calculated over the period between two harvests, increased with increasing light level ($P < 0.001$; Fig. 4.4a, Table 4.5). The NAR differed ($P < 0.05$) between biotypes but also depended on harvest time (Fig. 4.4b). The component LAR (m^2 g^{-1}(plant)) was very plastic and strongly increased with lower light levels ($P < 0.01$;Table 4.5). The differences ($P < 0.001$) in LAR between susceptible and resistant biotypes increased with decreasing light levels.

Table 4.4. The parameters c_m (maximum linear growth rate) and t_b (time of switch from exponential to linear growth rate) from the expolinear growth equation for the susceptible and resistant biotypes at different light levels. Standard errors in brackets.

PPFD (µmol m^{-2} s^{-1})	Biotype	Parameter c_m	t_b
133	Susceptible	3.14 (0.824)	28.39 (1.597)
213	Susceptible	1.49 (0.308)	23.42 (1.103)
377	Susceptible	3.69 (0.793)	22.27 (0.929)
133	Resistant	2.22 (0.726)	26.01 (1.913)
213	Resistant	3.90 (1.040)	27.28 (1.268)
377	Resistant	3.34 (0.427)	25.01 (0.975)

The Leaf area ratio was subdivided into LWR and SLA. The LWR (g (leaf) g^{-1} (plant)) hardly decreased with increasing light level and did not differ between biotypes (Table 4.5, Fig. 4.4b). Effect of population ($P < 0.05$) on LWR was very small and varied between harvests (significant interaction $P < 0.01$). The decline in LWR during the experiment indicated a lower investment of dry matter in leaves. At the same time, the shoot/root ratio, which was higher ($P < 0.001$) for the resistant biotype, increased slightly with time (Table 4.6), indicating a switch from investment in leaves to investment in stems and reproduction. This was supported by the fact that flowering (determined at each harvest) had started during the experiment. Flowering was delayed at lower light levels. The resistant seedlings started flowering later than the susceptible seedlings (Table 4.7). At the end of the experiment, biometric traits such as leaf area, number of forks and number of racemes were lower ($P < 0.01$) in resistant plants with the exception of plant height which did not significantly differ between biotypes (Table 4.8). Light level had an effect ($P < 0.01$) on leaf area and number of racemes.

Besides the LWR, the Leaf area ratio is also determined by the SLA (m^2 g^{-1}(leaf)). The SLA strongly decreased with increasing light level but this depended on the harvest time ($P < 0.01$, Fig. 4.5, Table 4.5). Specific leaf area was higher for the resistant biotypes at all light levels and harvests ($P < 0.001$) with a significant interaction between light and biotype ($P < 0.01$). The higher SLA of the resistant biotype points at a higher production of leaf area per unit dry leaf weight of the resistant biotype.

Discussion

Management of herbicide resistant weeds is an important topic in modern agriculture aiming at minimal use of, and dependence on, herbicides. Different strategies to prevent or manage herbicide resistance have been developed (Moss & Rubin 1993). In

Table 4.5. Significant effects of the experimental factors on RGR (total dm. total dm.$^{-1}$ d^{-1}), NAR (total dry matter leaf area^{-1} day^{-1}), LAR (leaf area total dry matter^{-1}) and LWR (dry matter leaf total dry matter^{-1}) analysed by ANOVA.

Factor	RGR	NAR	LAR	LWR	SLA
Light	***	***	**	*	**
Harvest	-	***	***	***	***
Population	-	-	-	*	-
Biotype	-	*	***	-	***
Light×Harvest	-	-	**	**	**
Light×Population	-	-	-	-	-
Light×Biotype	-	-	***	-	*
Harvest×Population	-	-	-	-	-
Harvest×Biotype	-	**	-	-	-
Population×Biotype	-	-	-	-	-

- not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

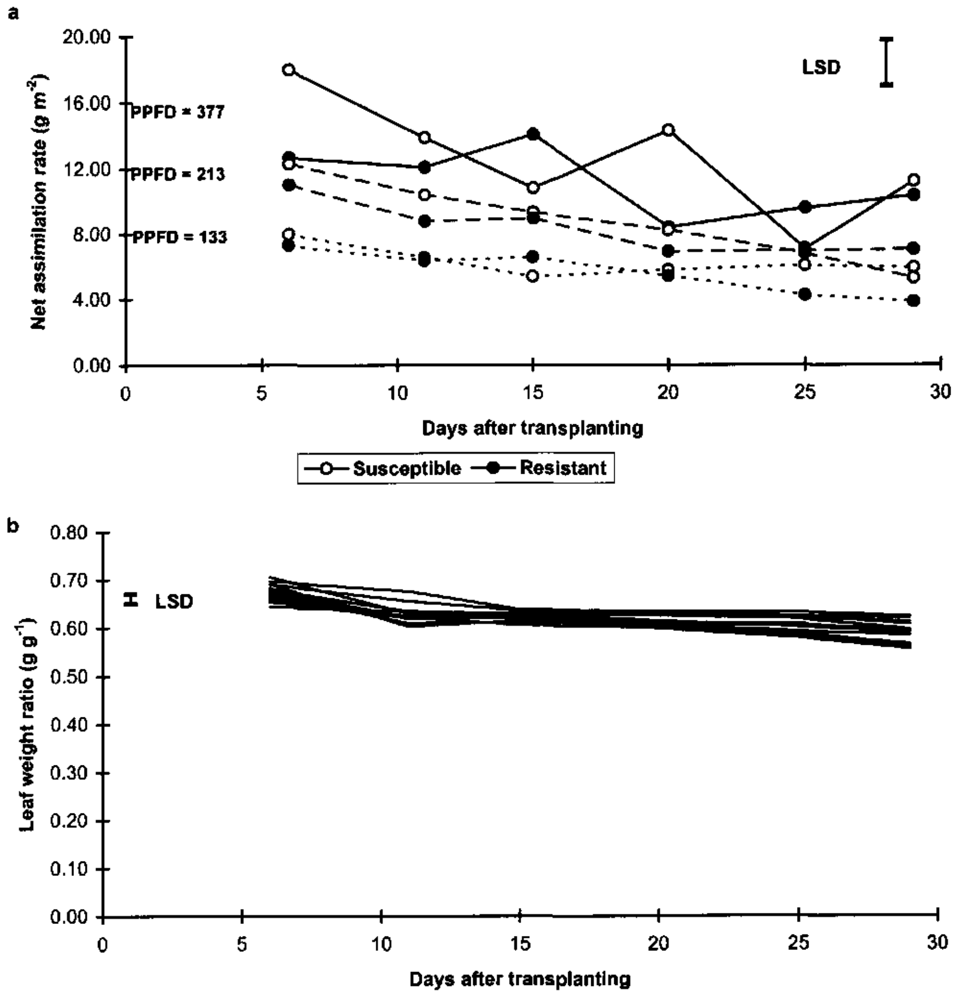

Fig. 4.4 (a) Net assimilation rate of the triazine susceptible and resistant biotype grown at three different light levels.

(b) Leaf weight ratio of different populations grown at different light levels which are not distinguishable.

the case of triazine resistant weeds, suggested management strategies are mainly based on fitness differences between biotypes in the absence of the herbicide. The magnitude of fitness differences between susceptible and resistant biotypes determines the selectivity of these management strategies. Relative fitness measures describe the potential evolutionary success of a genotype based on survival, competitive ability and ultimately reproductive success, with the most fit individual leaving the greatest number of offspring and thereby contributing a greater proportion of its genes to the gene pool of the population. Usually, fitness differences between susceptible and

Table 4.7. Onset of flowering of susceptible and resistant *S. nigrum* from different populations (days) determined at each harvest date.

Population	Photosynthetic Photon Flux density (μmol m^{-2} s^{-1})					
	133		213		377	
	Biotype					
	Susc.	Res.	Susc.	Res.	Susc.	Res.
Achterberg	20	25	15	25	15	20
Zelhem	20	25	15	25	15	20
Gorssel	15	25	15	25	15	15
Epse	20	25	20	20	15	20
Beerze	15	25	20	25	15	20

resistant biotypes are only characterized by plant productivity or competitiveness (Warwick & Black 1994).

Growth of triazine susceptible and resistant plants in the field has been studied in, amongst others, *Brassica napus* (Gressel & Ben-Sinai 1985) and *Amaranthus hybridus* (Ahrens & Stoller 1983, Jordan 1996). However, environmental conditions in the field are not controllable and extremely unpredictable making a clear interpretation of the experimental results very difficult. In a controlled environment, the variability is strongly reduced which permits a refined analysis of growth.

Growth experiments with individual *S. nigrum* plants in glasshouses showed a higher biomass production of the triazine susceptible biotype after 42 days (Domínguez *et al.* 1994) and 50 days (Bulcke *et al.* 1985) compared to the resistant biotype. In growth chambers, biomass production of susceptible biotypes was also higher than that of resistant biotypes after 30 days (Jacobs *et al.* 1988) and after 75 days (Zanin & Lucchin 1990). The corresponding results of growth in the young phase in these studies emphasize the importance of secondary effects of triazine resistance on growth.

In our experiment, the triazine susceptible biotypes of *S. nigrum* had a higher total biomass production than the resistant biotype which increased with increasing light levels. This supports the hypothesis of a negative effect of increasing light intensity on growth of the resistant biotype whereas the light levels in our experiment were still quite low (maximum PPFD 377 μmol m^{-2} s^{-1}). In *Brassica napus* (Hart *et al.* 1992) and *C. album* (Curwiel *et al.* 1993) an increased photoinhibition of the resistant biotype at

Table 4.8. Values of different biometric traits per biotype at the highest light level (376.5 (±2.3) μmol m^{-2} s^{-1}) at the final harvest, 29 days after the start of the experiment.

Growth parameter	Susceptible	Resistant
Plant height (cm)	18.8	17.1
Leaf area (m^2 $plant^{-1}$)	0.3501	0.2793
Number of forks	2.8	1.9
Number of racemes	10.1	6.4

higher light levels (max. PPFD of 1200 and 547μmol m^{-2} s^{-1}, respectively) resulted in reduced biomass production. Understanding the origin of these differences in final total biomass requires more detailed knowledge of growth processes of both biotypes at different light levels. Therefore, the data were analysed with an expolinear growth equation to detect underlying mechanisms in the current study.

The relative growth rate, determined by curve fitting, is the most stable parameter to compare growth of plants from different treatments during the exponential growth phase. To determine the relative growth rate, initial dry weights and periodic harvests are needed. In our case, the initial weights of the susceptible seedlings were higher than those of the resistant seedlings (Table 4.3) as was also found by Zanin and Lucchin (1990). Relative growth rates of the resistant biotypes were equal to those of the susceptible biotypes (Fig. 4.3) and no effect of photo-inhibition on the RGR of the resistant biotype at the highest light level was observed. Transition from exponential to linear growth took place earlier as light levels increased (Table 4.4) indicating that the period during which the RGR was applicable decreased with increasing light levels. This was expected as plants grow faster at higher light levels and mutual shading of leaves occurs at an earlier stage. Higher relative growth rates of resistant plants in the early growth stage have been reported by Zanin and Lucchin (1990), over the period 18-43 days after transplanting and by Domínguez *et al.* (1994, own calculations) over the period 21-35 days after germination. In our experiment, a difference in RGR between biotypes could have existed in the short period between emergence and transplanting but was not present during the course of our experiment.

The NAR (production per unit leaf area), calculated across adjacent harvest intervals, increased with increasing light levels and was slightly lower for the resistant biotype, independent of plant size. This could indicate the existence of an often reported reduced photosynthetic efficiency of the resistant biotype (Holt *et al.* 1993, Moss & Rubin 1993). Growth of triazine susceptible and resistant *S. vulgaris* in a glasshouse showed a equal NAR for both biotypes (Holt 1988) while Zanin and Lucchin (1990) found a higher NAR for resistant *S. nigrum* during early growth.

The LAR is a very plastic growth parameter and strongly affected by light (Jeangros & Nösberger 1992). Decreasing light intensities cause an increase in the LAR to maximize light capture of the plant (Brouwer 1962, Stoller & Myers 1989, Semb 1996). The significantly higher LAR of the resistant biotype of *S. nigrum* was also found in *S. vulgaris* by Holt (1988).

The LWR determines which fraction of the total dry matter of a plant is invested in leaves and declines with increasing light levels in *S. nigrum*. During the experiment, the LWR decreased while shoot/root ratio increased, implying a switch from investment in leaf biomass into investment in stems and reproduction. This is supported by the fact that flowering had started during the experiment. Flowering was delayed at lower light levels and resistant seedlings flowered later than susceptible seedlings, indicating a slower plant development of the resistant biotype. Differences in flowering time between biotypes ranged between 47.5 and 95 degree days, assuming

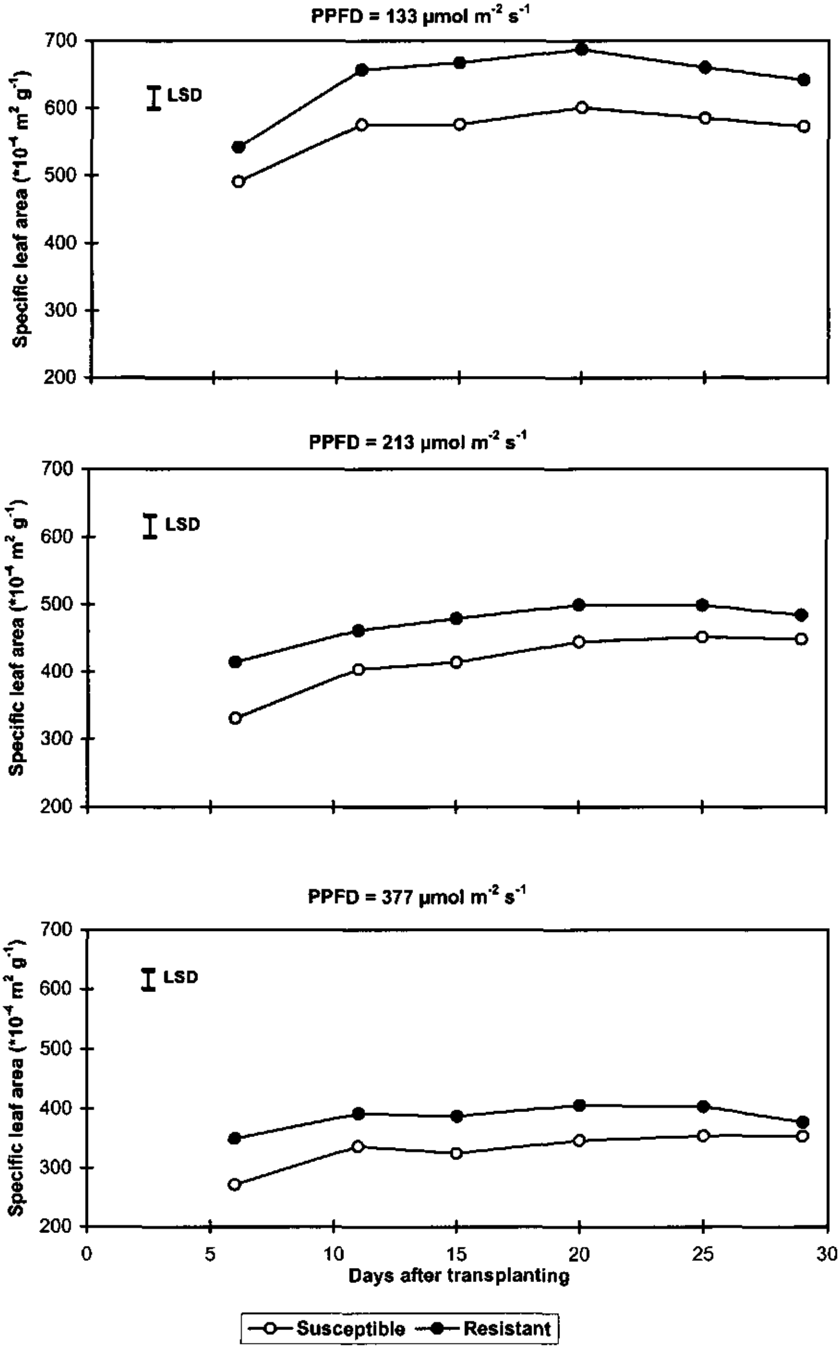

Fig. 4.5 Specific Leaf Area (cm^2 g^{-1} (leaf))of triazine susceptible and resistant *S. nigrum* grown at three light levels. Error bars indicate Least Significant Difference.

a base temperature of 6 °C for plant development (McGiffen & Masiunas 1992). Other biometric traits such as number of buds, number of forks and number of leaves differed among populations but were consistently lower for resistant biotypes, as reported before (Zanin & Lucchin 1990, Domínguez *et al.* 1994).

The most important component affecting LAR and thus determining RGR was SLA (Fig. 4.5). The higher SLA of the resistant biotype represented a higher leaf area production per unit dry leaf weight which decreased with increasing light levels. The SLA during early growth stages is determined by leaf expansion governed by temperature, and by dry matter growth which is mainly driven by radiation (Horie *et al.* 1979, Kropff & Van Laar 1993). The higher SLA of triazine resistant biotypes was also found for *S. vulgaris* by Holt (1988) and derived from results with *S. nigrum* of Domínguez *et al.* (1994). The higher SLA of the resistant biotype compared to that of the susceptible biotype is compensated by a lower NAR resulting in equal Relative Growth Rates for both biotypes.

Experiments with domesticated and wild plant species showed that differences in RGR at different light levels were highly correlated with LAR and much less with differences in NAR, biomass partitioning or photosynthesis per unit leaf area (Poorter & Remkes 1990, Van der Werf *et al.* 1996). Growth experiments with *Rumex obtusifolius* and *Lolium perenne* (Jeangros & Nösberger 1992) and Brussels sprouts and leek (Van der Werf *et al.* 1996) emphasized the importance of SLA in explaining RGR at different light levels.

The higher SLA of triazine resistant biotypes of *S. nigrum* may be a compensatory mechanism for the reduced photosynthetic efficiency. By increasing the leaf area per unit dry matter, photosynthesis per unit leaf area might be lower but overall photosynthesis per plant might be equal. Lemoine *et al.* (1986) found in triazine resistant *S. nigrum* shade-type chloroplasts. The adaptive reorganization of the thylakoid components seemed to be a compensatory mechanism for the reduced electron transport rate between Q_A and Q_B. Other secondary effects might exist and affect the fitness of triazine resistant biotypes.

The growth analysis showed an equal RGR of both biotypes where the SLA was distinctively higher for the resistant biotype compensated by a lower NAR. These findings under controlled conditions cannot directly be applied to field conditions. However, light levels under a closing maize crop are in the range of light levels in this experiment. Light quality, expressed as red/far red ratio, is also affected by the light interception of a crop (Morgan & Smith 1981). Red/far red ratios in the growth chamber ranged between 2.85 and 3.10 compared to a ratio of 0.3 under a maize crop, calculated from Sattin *et al.* (1994). Red/far red ratio mainly affects stem elongation and leaf dry weight/stem dry weight ratio (Morgan & Smith 1981).

The possible compensating effects of genome controlled traits on growth, potentially causing variable results, seem to be absent in this experiment with 5 populations. No effects of population on the growth components RGR, LAR, NAR and

SLA were observed (Table 4.5). Large differences in relative growth rates between triazine susceptible and resistant biotypes, as expected from earlier reports on growth, were distinctly absent in this experiment.

The observed equal RGR of dry matter suggests an equal competitive ability of the susceptible and resistant biotype under a crop in the absence of a triazine herbicide, resulting in equal dry matter production and reproduction. The difference in observed total final dry matter production between susceptible and resistant biotypes is most probably based on processes that already took place in the very young growth phase of the seedling. Since single plants in growth chambers and plants in the field grow differently it is necessary to evaluate growth patterns under field conditions and to get more information about the whole growth period.

The information about competitive ability obtained should be integrated with knowledge about secondary effects of triazine resistance on other processes in the life cycle e.g. dormancy and germination (Table 4.2; Kremer & Lotz 1998, Chapter 2). Knowledge of the complete life history can then be used to estimate fitness differences between biotypes to develop management strategies to control triazine resistant weeds.

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Chapter 5

Growth and reproduction of triazine susceptible and resistant *Solanum nigrum* in a maize crop

Abstract

Biomass and berry production of triazine susceptible and resistant *Solanum nigrum* in the field with and without a maize crop were studied during three years. Delayed transplanting reduced the final dry matter production and berry production strongly. In most of the experiments, the final total dry matter production and berry production of the triazine resistant biotype were lower than those of the susceptible biotype. However, relative growth rates of both biotypes in a maize crop were similar during the exponential growth phase. One important determinant of final dry matter and berry production could be dry matter growth in the very young seedling phase. Although dry matter production and reproduction of the resistant biotype in a maize crop will be lower than that of the susceptible biotype in the absence of the selecting herbicide, management strategies to control resistant biotypes of *S. nigrum* should not only be based on this characteristic.

Key-words: triazine resistance, black nightshade, competition, resistance management, population dynamics.

Introduction

Herbicide resistant weeds develop because of the selection pressure from continuous use of chemicals with the same mode of action. This is the case in The Netherlands, where forage maize (*Zea mays* L.) is generally grown continuously with yearly triazine applications. Triazine resistant weeds have been present in The Netherlands since 1982 (Van Oorschot & Straathof 1988). The resistance is based on a single gene mutation in the chloroplast which prevents inhibition of photosynthesis by the herbicide but also causes an impaired electron transport in photosystem II (Holt *et al.* 1993). The reduced electron transport might result in a lower photosynthetic activity and a lower fitness of the resistant biotype (Moss & Rubin 1993). A reduced fitness in the absence of the selecting herbicide is a very important characteristic in the development of strategies to manage triazine resistant weeds without the use of herbicides (Maxwell *et al.* 1990).

Differences in aspects of fitness between triazine susceptible and resistant weeds have been observed in many studies to date (Warwick & Black 1994). Determinations of differences in fitness were mainly focused on growth and seed production of susceptible and resistant weeds under competitive and non-competitive conditions. However, fitness not only involves growth and seed production, but also involves other life cycle characteristics such as seed survival, dormancy, germination, emergence, and development. Knowledge about secondary effects of triazine resistance on these processes is scarce.

Many studies on the growth characteristics of triazine susceptible and resistant biotypes of different weed species have been documented (reviewed by Holt & Thill 1994, Warwick & Black 1994). Generally, the susceptible biotypes had a lower biomass and seed production but results were often equivocal. Many experiments did not include isogenic lines, or biotypes from the same field, or were performed in glasshouses under growth-limiting conditions (Holt 1997). A correct comparison of growth should be made under optimal growth conditions, using populations with both susceptible and resistant biotypes that come from several geographical origins to determine the variation in growth parameters within and between populations.

An earlier study of the exponential growth phase of susceptible and resistant biotypes from five populations of different geographical origin was carried out in a growth chamber where growth was only limited by light. Results showed an equal relative growth rate (RGR) of susceptible and resistant biotypes at the different applied light levels in all populations (Chapter 4). In that experiment, the final total dry matter of the plants after 29 days was primarily determined by initial seedling weight at the start of the experiment.

Growth in a growth chamber or glasshouse does not reflect the interacting stresses in a field cropping situation. In the field, the weather is variable, light quality and quantity continuously fluctuate or change, and the weeds compete with the crop. Results from experiments in growth chambers or glasshouses should be validated under field conditions before any final conclusions about growth in the field can be drawn (Jordan 1996).

Four experiments were performed in different years at different sites in The Netherlands. In the first year, the effect of date of transplanting of young *S. nigrum* seedlings on growth of susceptible and resistant biotypes in a maize crop was studied. In the subsequent two years, dry matter production of susceptible and resistant biotypes at the end of the season in presence or absence of the maize crop was studied with only one planting date of pre-germinated seeds.

The objective of these experiments was to compare the final total dry matter and berry production of triazine susceptible and resistant biotypes of *S. nigrum* in the presence or absence of a maize crop and test whether relative growth rates in the exponential growth phase could explain possible differences.

Material and methods

Experiment 1 (1994)

The effect of time of establishment on growth of triazine susceptible and resistant biotypes of *S. nigrum* in a maize crop was studied in an experiment where transplanting date was varied. Seeds from triazine susceptible and resistant plants of one population from one field in Achterberg, The Netherlands (51°59' N, 5°35' E), identified by fluorescence measurements, were pre-germinated and seedlings were

Table 5.1 Experimental data of experiment 1.

Experimental data			
Emergence date maize crop	14 May 1994		
Light level under closed crop (μmol m^{-2} s^{-1}) (crop/full light ratio)	21 September: 116/961		
Transplanting time seedlings (days after crop emergence)	3	38	79
Harvest dates of *S. nigrum* (days after transplanting)	42, 69, 128	48, 71, 108	51, 73

grown in a glasshouse up to the 2-4 true leaf stage. The seedlings were transplanted into a maize field on sandy soil at different dates (Table 5.1). Crop row spacing was 0.75 m and plots consisted of the area between two crop rows over a length of 5 m. Per plot, 20 seedlings of one biotype were planted alternately in two rows 25 cm apart. No interaction between plants was observed during the season. The plots were kept free from other weeds by handweeding. The treatments were replicated four times in a randomized block design.

Several harvests were performed during the season (Table 5.1). At each harvest, plant height, number of forks, branches, flowers, unripe berries, and ripe berries were determined as well as dry matter of leaves, stems and remaining berries per plant. The sympodial flowering of *S. nigrum* resulted in ripe berries which already fell off during the season. Empty spots were counted and multiplied with a ripe berry dry weight of 0.07 g (pers. obs.) to account for missing berries in the final total dry matter production.

Median values of parameters of every plot were analysed using multiple linear regression on single harvests. The number of harvested plants per plot varied and was used as a weighting factor to account for missing plants (Payne *et al.* 1987). RGR (Relative Growth Rate; total dm total dm^{-1} d^{-1}) was calculated with a linear regression on the natural logarithm of total dry matter of the plant. RGR was determined during the exponential growth phase in the period 0-69 days after transplanting date 1 and 0-48 days after transplanting date 2.

Experiment 2 (1995)

Growth of young triazine susceptible and resistant *S. nigrum* plants in the exponential growth phase was studied in a maize crop in a short experiment with four intermediate harvests. Pre-germinated seeds of triazine susceptible and resistant biotypes of *S. nigrum* from the Achterberg population were sown in a maize field on a sandy soil (Table 5.2). Plots consisted of an area covering two maize rows over a length of 2 m with 8 planting spots (1.5 m x 2 m) and were kept free from other weeds by handweeding. Seedlings of one biotype were planted alternately in two rows 25 cm apart between the crop rows.

Harvests were performed at 9, 18, 25, and 32 days after emergence of the weed to determine RGR during the exponential growth phase. At each harvest, leaf area, dry matter of leaves, and dry matter of stems were measured. RGR was determined by linear regression on the natural logarithm of the median value of total above-ground dry matter per plant with number of harvested plants per plot as weighting factor. Experimental set-up consisted of a randomized block design with 4 replicates.

Experiment 3 (1995)

The effect of presence of a maize crop on growth of triazine susceptible and resistant biotypes during the season was studied in 1995 with one planting date and one final harvest. Pre-germinated seeds of triazine susceptible and resistant biotypes of *S. nigrum* from the Achterberg population and susceptible and resistant biotypes of one population from Zelhem, The Netherlands (51°59' N, 6°18' E) were sown in the same maize field as in experiment 2 (Table 5.2). Plot size was 1.5 m x 4 m covering 2 crop rows with 32 planting spots. Per plot, the seedlings of one biotype from one population were planted alternately in two rows 25 cm apart between the crop rows. Plots were kept free from other weeds by handweeding; no interaction between *S. nigrum* plants was observed. Plant density and leaf area development of the maize was determined by harvesting 2 m of the crop row and measuring leaf area. Plots without a crop had a size of 3.75 m x 4 m, the maize plants had been removed to avoid competition. The plant density of *S. nigrum* was 5.3 plants per m^2 and the plot was kept free from other weeds by handweeding.

During the season, on 8 August, the morphology of the *S. nigrum* plants was determined by measuring the height, number of leaves, number of forks, number of flowers, and number of berries of all plants in the experiment non-destructively. At the final harvest at the end of the season, total dry matter of stems, leaves, and remaining berries was determined as well as the number of berries and the number of fallen ripe berries. The total above-ground dry matter production included an account for missing berries. The experiment was set up in a randomized block design with 4 replicates. The data were analysed separately for the monoculture of *S. nigrum* and the weed-crop mixture, using multiple linear regression analysis on the median values of the parameters per plot with the number of harvested plants per plot as weighting factor.

Table 5.2 Experimental data of the experiments 2, 3 and 4.

	Exp. 2 (1995)	Exp. 3 (1995)	Exp. 4 (1996)
Emergence date maize crop	28 May	28 May	12 May
Emergence date *S. nigrum*	21 June	21 June	24 May
Crop canopy characteristics	Fig. 5.2a	Fig. 5.2a	Fig. 5.2b
Light level under closed crop	14 September:	14 September:	7 October:
(μmol m^{-2} s^{-1}) (crop/full light ratio)	24/601	24/601	43/610
Intermediate harvests	3	-	-
Start final harvest	28 July	16 October	30 September

Experiment 4 (1996)

The effect of presence of a crop on growth of triazine susceptible and resistant biotypes was studied again in 1996. Pre-germinated seeds of triazine susceptible and resistant biotypes of *S. nigrum* from the Achterberg population and from the Zelhem population were sown in a maize field (Table 5.2). Plot size in the crop was 1.5 m x 4 m with 16 planting spots while the monoculture plots (where maize plants had been removed) had an area of 6.25 m x 5 m with 12 planting spots. In the crop, seedlings of one biotype were planted alternately in two rows 25 cm apart between the crop rows; no interaction was observed during the season. The monoculture weed plots had a square plant distribution with a spacing of 1.25 m preventing interactions between individual plants. The field was irrigated during the season to ensure optimal growing conditions and was kept weed-free by handweeding. Plant density and leaf area development of the maize was determined by harvesting 2 m of the crop row and measuring leaf area.

The weeds were harvested only once at the end of the growing season. The enormous size of the isolated weed plants necessitated the partial harvest of 4 plants per plot only. Based on the percentage of total fresh weight used in the harvest, data were translated to the whole plant level. All *S. nigrum* plants grown in mixture with the crop were harvested. During the final harvest, total above-ground dry weight of plants, dry weight of remaining berries and the total number of berries per plant were determined. Total dry matter production included an account for missing berries. At the same time, number of seeds per berry and thousand seed weights were assessed. Multiplication of the total number of berries per population and biotype with the number of seeds per berry resulted in the fecundity index (number of seeds per gram dry matter plant). The experiment was set-up as a randomized block design with 4 replicates. Data were analysed separately for the *S. nigrum* plants grown separately and those grown under the crop canopy, with a multiple linear regression analysis on the average value of the 4 harvested plants of the monoculture and on the median values of the parameters of plants grown in mixture with the maize crop with the number of harvested plants per plot as weighting factor.

Results

In experiment 1, the total dry matter production of susceptible plants from the Achterberg population was only higher than that of resistant plants at the transplanting dates and a few individual harvests ($P < 0.05$, Fig. 5.1) where the analysis included the variable number of harvested plants per plot as a weighting factor. Total berry production differed only between biotypes at the first harvest after planting date 1 where the resistant biotype produced fewer berries and at the first harvest after planting date 3 where the resistant biotype produced more berries ($P < 0.05$). A delay in transplanting date caused a reduction in final total dry matter and total berry production at the end of the season. The relative growth rates of both biotypes in a maize crop during the exponential growth phase were similar. Between transplanting date 1 and harvest 2, RGR's of the susceptible and resistant biotype had values of date

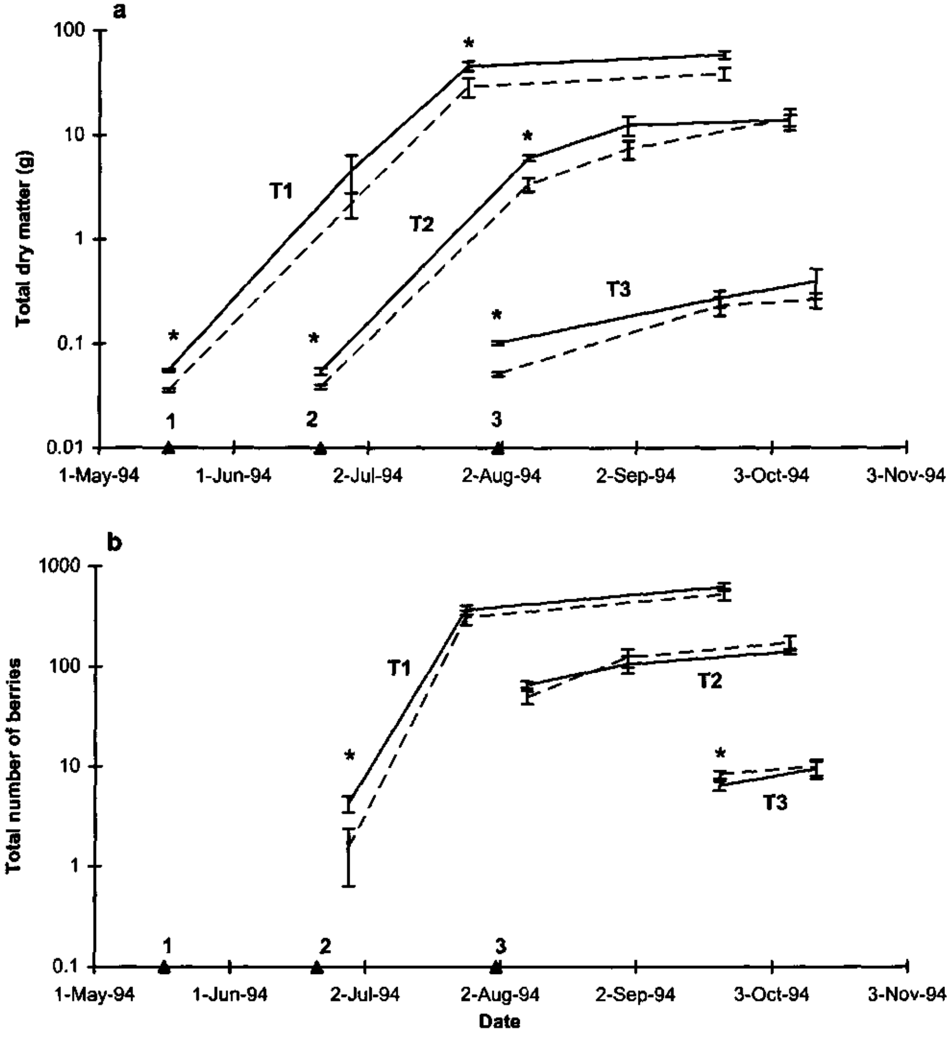

Fig. 5.1 Development of dry matter (a) and number of berries (b) per plant of susceptible (———) and resistant (– – – –) *S. nigrum* plants in experiment 1 at three different transplanting dates (T1, T2, and T3). Asterisks indicate significant differences ($P < 0.05$) between biotypes at single harvests. Triangles on the x-axis indicate transplanting dates and vertical bars indicate two times the standard error.

1 and harvest 2, RGRs of the susceptible and resistant biotype had values of 0.097 and 0.096, respectively. RGRs of the susceptible and resistant biotype between transplanting date 2 and harvest 1 had values of 0.098 and 0.092, respectively. Susceptible and resistant seedlings, transplanted 35 days later in a developing maize crop (transplanting date 2) produced respectively 75% and 61% less dry matter. Plants from

transplanting date 3 (76 days later) accumulated only 1% dry matter compared to that of plants from transplanting date 1.

In experiment 2, the leaf area development of maize was measured in the first part of the season and expressed as Leaf Area Index (m^2 leaf m^{-2} soil; Fig. 5.2). The LAI increased exponentially in the first part of the season and seemed to have reached a maximum of approximately 4 in August. Total dry matter production of the susceptible biotype was higher than that of the resistant biotype up to 32 days after seedling emergence ($P < 0.05$) with the exception of the third harvest (Fig. 5.3). However, the relative growth rate of the susceptible biotype (0.154) was equal to that of the resistant biotype (0.156). No berries were produced during the experiment.

In experiment 3, two populations from different geographical origins (Achterberg and Zelhem), both including a susceptible and a resistant biotype, were involved in the comparison of growth of susceptible and resistant biotypes in presence or absence of a maize crop. At the intermediate non-destructive harvest, presence of a crop considerably reduced the level of all parameters ($P < 0.001$; Table 5.3). In the crop, plants from the Zelhem population were lower and had more berries than plants of population Achterberg ($P < 0.05$). The resistant biotypes had a lower plant height ($P < 0.001$), number of forks ($P < 0.001$), and number of flowers ($P < 0.01$) than the susceptible biotypes. The resistant biotypes also had a lower number of leaves than the susceptible biotypes but an interaction with the presence of a crop was observed

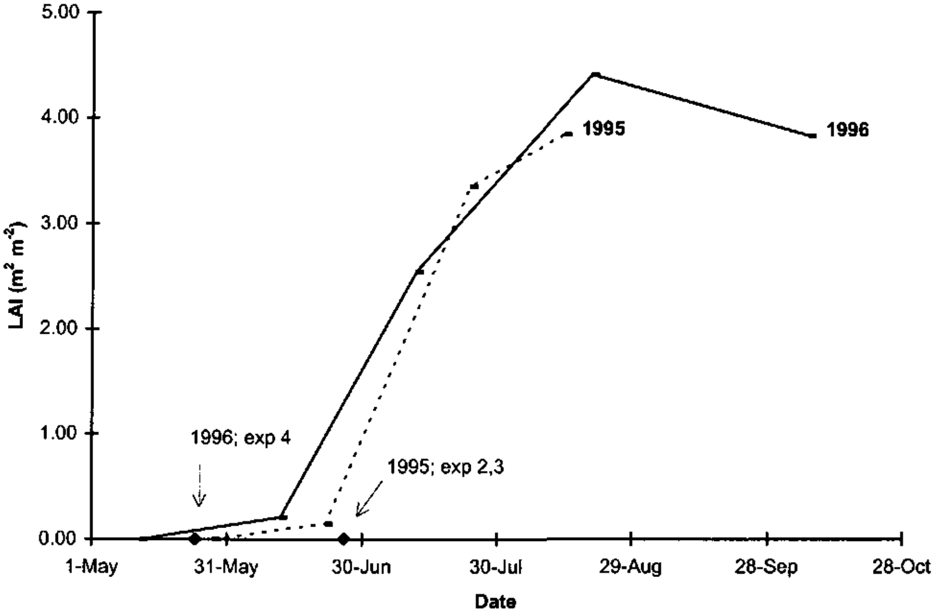

Fig. 5.2 Leaf area development of the maize crop, expressed as LAI, in experiment 2, 3, and 4. Symbols on the x-axis indicate emergence time of *S. nigrum*.

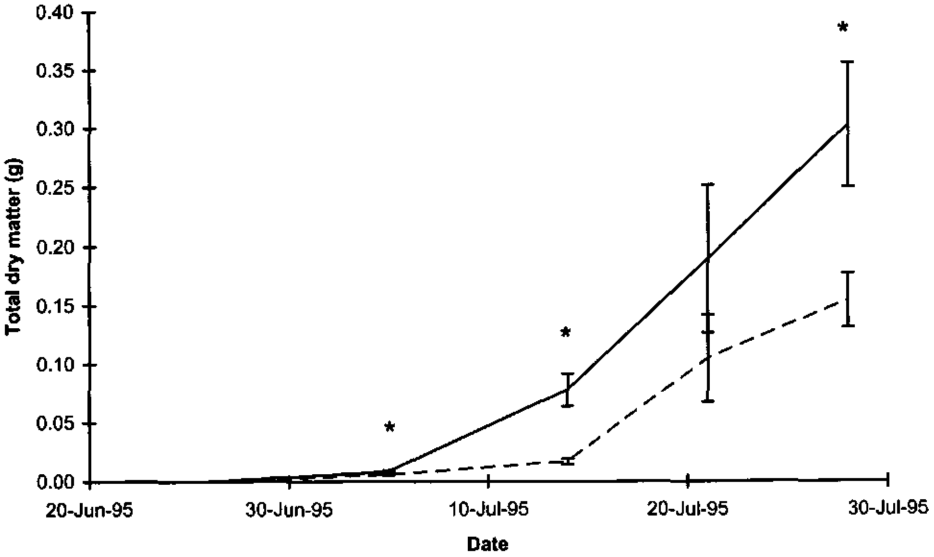

Fig. 5.3 Above-ground dry matter production per plant in time of triazine susceptible (——) and resistant (– – –) seedlings in the exponential growth phase in a maize crop in experiment 2. Asterisks indicate significant differences ($P < 0.05$) between biotypes at single harvests.

Table 5.3. Morphology of susceptible and resistant biotypes of *Solanum nigrum* from populations with different geographical origins determined at the non-destructive harvest during experiment 3 (8 August 1995) with standard errors in parentheses.

Parameter	Population	Achterberg		Zelhem	
		Susceptible	Resistant	Susceptible	Resistant
Height (cm)	Pure weed stand	46.0 (1.8)	41.0 (3.3)	49.6 (2.1)	38.8 (1.8)
	Maize weed mixture	24.9 (4.0)	15.3 (2.2)	19.9 (1.3)	13.8 (1.9)
No of leaves	Pure weed stand	230.1 (2.5)	197.3 (22.7)	314.9 (60.3)	178.4 (14.8)
	Maize weed mixture	16.4 (0.8)	11.5 (0.9)	13.6 (2.1)	11.4 (0.8)
No of forks	Pure weed stand	5.5 (0.3)	4.3 (0.3)	6.0 (0.4)	3.9 (0.3)
	Maize weed mixture	2.3 (0.3)	1.4 (0.2)	2.3 (0.5)	1.3 (0.3)
Flower no	Pure weed stand	28.9 (1.5)	22.3 (7.2)	50.9 (2.2)	25.4 (5.8)
	Maize weed mixture	5.5 (0.5)	2.3 (0.8)	6.0 (0.7)	2.0 (0.9)
Berrie no	Pure weed stand	12.6 (1.3)	4.0 (0.5)	37.9 (2.6)	10.1 (2.9)
	Maize weed mixture	3.4 (1.2)	0.0 (-)	4.3 (2.3)	0.3 (0.3)

Table 5.4. Total dry matter production (g plant^{-1}) and total number of berries per plant at the final harvest in different experiments in pure weed stands (pure) and mixed stands (mixed). Standard errors in parentheses. Asterisks indicate a significant difference between biotypes. In experiment 3, a significant effect ($P < 0.05$) of population was observed on dry matter production in mixed stands.

Exp. and Population	Dry matter		Number of berries	
	Susceptible	Resistant	Susceptible	Resistant
Exp. 1				
Mixed, Achterberg	58.58 (5.02)	38.58 (5.40)	618 (53)	516 (66)
Exp. 3				
Pure, Achterberg	217.5 (27.2)	189.4 (22.8)	1823 (315)	2072 (249)
Pure, Zelhem	224.7 (44.5)	157.0 (36.4)	2236 (434)	1577 (266)
Mixed, Achterberg	2.23 (0.43)	1.18 (0.27) *	23.4 (4.2)	16.8 (2.9)*
Mixed, Zelhem	1.31 (0.51)	0.85 (0.11) *	16.1 (6.1)	11.8 (1.9)*
Exp. 4				
Pure, Achterberg	1079.1 (109.4)	642.4 (68.0) **	9682 (1491)	7734 (915) *
Pure, Zelhem	955.8 (136.6)	748.1 (59.5) **	10827 (1363)	8342 (617) *
Mixed, Achterberg	52.13 (8.08)	22.35 (2.47) **	510 (59)	329 (41) *
Mixed, Zelhem	32.00 (7.11)	21.97 (4.93) **	395 (81)	342 (68) *

* $P < 0.05$; ** $P < 0.01$

($P < 0.01$). Berry production was affected by population, biotype, presence of a crop and the interactions between these factors ($P < 0.01$).

The most important parameters characterizing growth and reproduction at the end of the season in experiment 3 are represented in Table 5.4. Total dry matter production and number of berries in the pure stand did not differ between biotypes and populations. The difference in dry matter production between biotypes when grown in mixture with the crop depended on the population and was 47 % lower for the resistant plants from Achterberg and 35 % lower for the resistant plants from Zelhem compared to susceptible plants from the same population ($P < 0.05$). Resistant plants produced 27 % fewer berries than the susceptible plants when grown in the maize crop ($P < 0.05$).

In experiment 4, the LAI increased during the season to a maximum of 4.5 at the end of August (Fig. 5.2). Senescence of leaves resulted in a reduced LAI later in the season. In the irrigated experimental field, final dry matter and berry production were considerably higher in 1996 than in 1995 (Table 5.4). In the pure weed stand, the dry matter production of the resistant biotypes of *S. nigrum* was 32 % lower than that of the susceptible biotypes ($P < 0.01$). Berry production of the resistant biotype was on average 22 % lower than that of the susceptible biotype ($P < 0.05$). Grown in the maize crop, the dry matter production of the resistant biotype was 48 % lower than that of the susceptible biotype ($P < 0.01$). The berry production of the resistant biotype in the

Table 5.5. Number of seeds per berry and seed weights of the susceptible and resistant biotype grown in pure weed stands or mixed with a crop in experiment 4. Standard errors in parentheses.

	Number of seeds/berry		Thousand seed weight (g)	
	Susceptible	Resistant	Susceptible	Resistant
Pure	68.1 (2.6)	64.9 (1.1)	0.8812 (0.0134)	0.8163 (0.0144)
Mixed	64.0 (1.3)	56.5 (1.0)	0.6607 (0.0171)	0.6774 (0.0146)

maize crop was 26 % lower than that of the susceptible biotype ($P < 0.05$). No significant effect of population on total dry matter or berry production was observed in experiment 4.

The number of seeds per berry and the seed weight were also determined in experiment 4 (Table 5.5). The number of seeds per berry was higher in the pure stand than in the mixture and higher in the susceptible biotype compared to the resistant biotype ($P < 0.001$). The difference in number of seeds per berry between biotypes increased when plants were grown in mixture with the crop compared to pure stands. The seed weight was much higher in the pure weed stands than in the mixture ($P < 0.001$). No main effect of biotype on seed weight was observed while there was an interaction between presence of a crop and biotype resulting in a higher thousand seed weight of the susceptible biotype in the monoculture and similar seed weights of both biotypes in the mixture.

To correlate total dry matter production and seed production per plant, the fecundity index (number of seeds / g dry matter plant) was analysed in 1996. In pure stands, the fecundity index was higher for the resistant biotype of Achterberg compared with the susceptible biotype ($P < 0.05$), while the fecundity index was similar for both biotypes of the Zelhem population (Table 5.6). In the cropping situation, the fecundity index was significantly higher for the resistant biotype ($P < 0.05$) in both populations (Table 5.6).

Since the number of berries produced per plant and the number of seeds per berry were known, reproductive output per plant could also be estimated in experiment 4. In pure stands, without competition, the susceptible biotype produced 698,331 seeds per plant and the resistant biotype produced 521,666 seeds per plant. In the crop, the susceptible biotype produced 28,960 seeds per plant and the resistant biotype produced 18,956 seeds per plant. Presence of a crop reduced the seed production of the

Table 5.6. Fecundity index (number of seeds per gram total dry matter per plant) of the two populations with both biotypes grown in the field in experiment 4. Standard error in parentheses.

	Achterberg		Zelhem	
	Susceptible	Resistant	Susceptible	Resistant
Pure	1273 (69.9)	1722 (52.2)	1807 (84.5)	1682 (40.0)
Mixed	1169 (88.3)	1587 (87.6)	1399 (100.1)	1698 (101.8)

susceptible and resistant biotype by 95.9% and 96.4%, respectively, compared with the pure stand.

Discussion

The analysis of differences in final total dry matter production of triazine susceptible and resistant biotypes of *S. nigrum* in a crop requires knowledge about the growth pattern during the growing season. Competition by the crop has a strong effect on growth of weeds and a delayed emergence of the weed compared to that of the cropresults in a strong reduction in total dry matter and berry production (Fig. 5.1, Kropff & Van Laar 1993). The negative effect of a delayed planting date on growth of *S. nigrum* in pure stands was observed by Keeley and Thullen (1983). A delay in transplanting of *Solanum ptycanthum* in a 75-cm row soybean crop with 3 or 6 weeks showed a reduction in dry weight of 66% and 97% respectively (Stoller & Myers 1989).

The higher total dry matter production of the susceptible biotype compared to that of the resistant biotype in experiment 4 was independent of population. The variation between populations from different geographical origins was smaller than between biotypes within populations. Ahrens and Stoller (1983) also found in *Amaranthus hybridus* a lower above ground dry weight of the resistant biotype than the susceptible biotype when grown in pure stands in the field, but origin of both biotypes was not well-documented. Jordan (1996) found a reduced growth of the resistant biotype of *A. hybridus* in the field but the magnitude of the differences with the susceptible biotype depended on the year and on the populations from different geographical origins. Gray *et al.* (1995) found a similar dry shoot biomass production for susceptible and resistant biotypes of *Abutilon theophrasti* in the field under non-competitive conditions. Biotypes came from different geographical origins and in this rare case, triazine resistance was based on enhanced glutathione conjugation of atrazine. In general, biotypes with a triazine resistance based on the mutation in the chloroplast showed a reduced dry matter production in the field (Holt & Thill 1994).

The reproductive effort of both biotypes was estimated by the fecundity index (number of seeds / g total dry matter plant). The effect of population on fecundity index in the pure stands, may have had to do something with the size of the plants since larger plants might have invested a relatively higher fraction of their dry matter in supporting tissue. The higher fecundity index of the resistant biotype grown in mixture with a crop was especially related to more berries per gram total plant dry weight (not shown) while the number of seeds per berry was lower. This could indicate an increased investment of resistant plants in reproductive output when growing in a crop, compensating a possible reduced photosynthetic capacity.

Differences in growth between triazine susceptible and resistant biotypes in a crop can be affected by two resistance related processes. Hart *et al.* (1992) and Curwiel *et al.* (1993) found in respectively *Brassica napus* and *Chenopodium album* a negative effect of photo-inhibition on growth of resistant plants at higher light levels. In our experiments, the very open maize crop in the first part of the growing season could have resulted in a higher sensitivity to photo-inhibition of the resistant biotype. Besides that, the resistant biotype has a reduced electron transport rate in photosystem II (Holt 1988, De Prado *et al.* 1992). If this implicates an impairment of physiological functions, a closed maize crop canopy with reduced light levels underneath it, might further limit growth of the resistant biotype compared to that of the susceptible biotype (Jordan 1996).

The RGR of susceptible and resistant biotypes in experiment 1 after transplanting date 1 and 2 was similar but the transplanting stress experienced by the seedlings might have been different for the different transplanting dates and the timing of the harvests might have affected the calculated RGR values. In the second experiment, RGR of young plants in the exponential growth phase, in a maize crop, showed no differences between the susceptible and resistant biotype as well. In both experiments, differences in final total dry matter production were caused by other factors than the RGR.

The differences in final total dry matter production between susceptible and resistant biotypes seem to increase when plants were grown in mixture with the maize crop compared to the monoculture *S. nigrum* (Table 5.4). This could indicate a reduced photosynthetic activity of the resistant biotype compared to the susceptible biotype which effect would be more severe under the limiting light levels experienced under a crop canopy. However, since a maize crop is very open during its initial growth stages and relative growth rates of both biotypes were similar, this supposed reduction seems not to play a crucial role in the reduced final total dry matter of the resistant biotype. Therefore, a possible increased sensitivity to photo-inhibition at higher light levels of the resistant biotype compared to the susceptible biotype seems to be of more importance. Similar emergence dates of the crop and the *S. nigrum* plants in 1995 and 1996 resulted in high light levels endured by the seedlings at the start of the season, similar to those in the pure stands (Fig. 5.2). A possible negative effect of photo-inhibition on growth of the resistant seedlings in the early phase, combined with similar relative growth rates of both biotypes could have caused the differences in final total dry matter production as was observed in the growth chamber (Chapter 4).

Jordan (1996) proposed the increase of crop interference to reduce the dry matter production of triazine resistant biotypes. Williams *et al.* (1995) also observed an increasing cost of resistance in transplanted triazine susceptible and resistant *Datura stramonium* when grown in a maize crop. This agrees with the reduced dry matter production of triazine resistant *S. nigrum* in maize observed in our experiments but the susceptible and resistant biotypes seemed to have similar relative growth rates in a maize crop. While the crop has a negative effect on dry matter production of *S. nigrum*, biomass production of susceptible and resistant biotypes immediately after emergence (or before transplanting in an experiment) seemed to have a much higher impact on final total dry matter accumulation than differences in growth between biotypes.

A possible negative effect of photo-inhibition on seedling growth of the resistant biotype might have implications for the management of triazine resistant *S. nigrum*.

Since the resistant seeds have a lower minimum germination temperature requirement (Kremer & Lotz 1998, Chapter 2), resistant seedlings may emerge earlier in the season. This gives good options for control by a stale seedbed preparation or a non-selective herbicide. However, if the seedlings are not controlled, growth of the resistant seedlings might be reduced by photoinhibition early in the season. This will result in a decreased dry matter and seed production of resistant plants at the end of the season which will not be compensated by the headstart in growth due to earlier emergence (Fig. 5.1). In a weed management strategy with the aim to control triazine resistance, this means that resistant plants which escape weed control will have a lower reproductive output than susceptible plants but will still remain in the population. Control of resistant populations should be achieved by a combination of this knowledge and techniques which interfere with other parts of the life cycle of triazine susceptible and resistant *S. nigrum*.

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Chapter 6

Seed survival of triazine susceptible and resistant *Solanum nigrum*

Abstract

The fate of seeds from triazine susceptible and resistant *S. nigrum* in the soil was studied during the growing season and outside the growing season. The highest mortality occurred during the growing season after which only 28% of the resistant seeds and 48 % of the susceptible seeds remained viable. The number of emerged seedlings was similar for both biotypes. The highest difference in seed mortality between biotypes occurred at depths between 6 and 15 cm. The difference between the two biotypes could probably be partly attributed to more fatal germination of the resistant seeds because of its lower minimum germination temperature compared to that of the susceptible seeds, but more factors were involved. Seed mortality outside the growing season was lower than 4 %. The large differences in seed mortality between resistant and susceptible biotypes during the year could have implications for the population dynamics and development of management strategies to control triazine resistant weed populations.

Key-words: triazine resistance, black nighshade, seed bank, longevity, resistance management.

Introduction

Solanum nigrum is an important annual weed in the continuous cropping of maize in The Netherlands. Its success is based on the persistent seed bank and the emergence in late spring and summer, resulting in inadequate control by a contact herbicide (Weller & Phipps 1979). The continuous use of triazine herbicides, a soil applied herbicide, in maize crops has resulted in the development of triazine resistant biotypes of *S. nigrum*. Triazine resistant biotypes have a lower fitness than susceptible biotypes and this might offer possibilities to develop management strategies to control triazine resistant weeds (Matthews 1994, Kremer & Lotz 1998a, Chapter 2). To analyse these differences in fitness between susceptible and resistant biotypes and use them in an effective way, more knowledge about the life history and ecology of triazine susceptible and resistant biotypes of *S. nigrum* is required. The survival of seeds in the soil is an important aspect of the life history of weeds (Cousens & Mortimer 1995).

In arable fields, the majority of weed species are annuals and the seed bank is the major source for the renewal of weed plant populations (Dessaint *et al.* 1997). In general, weed seed banks are dominated by a few species only. These species persist in cropping systems because they are able to withstand control measures and adapt to crop management practices (Buhler *et al.* 1997). The density of the weed seed bank varies greatly in space and time and is closely linked to the cropping history of the field (Wilson 1988). The major input of the seed bank comes from seed producing weed plants in the same field. Many weed species have the potential for prolific seed production which is, in a cropping system, reduced by competition from the crop, herbicide damage and other management factors. A part of the weed seeds in the seed bank will survive and stay dormant in the soil for some years depending on the species, depth of seed burial, soil type, and tillage (Wilson 1988).

Gressel and Segel (1990) reported on the importance of seed bank dynamics for the development of resistant weed populations. From their model, they concluded that an increased persistence of seeds in the soil resulted in a larger buffering effect of seeds from previous years, decreasing the rate of evolution of resistance. Lower persistence of resistant seeds might also result in a faster exhaustion of the resistant seed bank when seed input is prevented.

Seeds in the soil can be subject to three processes which are important for maintenance of the population, namely germination, dormancy, and mortality. When a seed germinates and the seedling successfully emerges, it adds a potential seed producer to the population which further increases the population size. The dormancy state of seeds is important for the long term dynamics of the weed population, dormant seeds will remain viable for a longer time than non-dormant seeds. The mortality of seeds in the soil is an important aspect of different weed control options (Burnside *et al.* 1996). Mortality may consist of germination without successful emergence, predation, or degradation. Increasing the death rate of the seeds while seed input is prevented will further reduce the population size of the weed.

The mortality rate of seeds in the seed bank is affected by a variety of environmental changes brought about by seasonal climatic changes, tillage, irrigation, and cropping schemes. This implies that farming practices can significantly contribute to the manipulation of the seed bank (Zorner *et al.* 1984) and even may be helpful in the control of resistant weed populations (Matthews 1994). These farming practices can include a variable depth of tillage (Zorner *et al.* 1984) or timing of seedbed preparation (Thompson *et al.* 1994, Kremer & Lotz 1998a). According to Dyer (1995), the depletion of weed seed banks through interfering with dormancy or germination requirements has strong potential to enhance weed management options in agricultural systems.

A difference in longevity of the soil seed bank between triazine susceptible and resistant biotypes of *S. nigrum* could offer opportunities to exhaust the soil seed bank by changing management practices (Watson *et al.* 1987). Seed bank exhaustion is based on a combination of a reduced seed production of weed plants and a smaller seed bank by changing tillage systems which affects the vertical distribution and density of the weed seed bank (Buhler 1995).

The objective of this study was to determine the survival of triazine susceptible and resistant seeds in the soil over time as a part of fitness of both biotypes which is supposed to be lower for the resistant biotype compared to that of the susceptible biotype. Possibilities to use this insight for the development of management strategies to control triazine resistant weeds are discussed.

Material and methods

Experiment 1

The survival of triazine susceptible and resistant seeds of *S. nigrum* was determined during the growing season of 1995. Triazine susceptible and resistant *Solanum nigrum* plants from a population from Achterberg, The Netherlands (51°59' N, 5°35' E) were grown in the glasshouse for seed production in 1994. Triazine resistance of plants was confirmed using measurements of quantum yield based on the fact that resistant plants have a lower photosynthetic activity (Domínguez *et al.* 1994). Berries were collected from 48 plants per biotype and seeds were extracted by washing and sieving after which the seeds were dried. In December 1994, batches of 3000 seeds were buried in nylon bags in the field at a depth of 5 cm for stratification. On 4 May 1995, PVC cylinders with a diameter of 30 cm and a height of 25 cm were dug in a sandy soil which originally contained no *S. nigrum* seeds. The soil from each cylinder was very thoroughly mixed with seed batches from exhumed bags with a known biotype and a known number of viable seeds, and put back in the cylinder in the field. Design consisted of a randomized block design with three replicates and two treatments (susceptible or resistant). One cylinder was filled with soil without seeds to measure the soil temperature at depths of 25, 20, 15, 10, 5, and 1 cm. During the summer, emerged seedlings were counted and removed every few days. In October, at the end of the growing season, soil was exhumed from the cylinders in layers with different depths and thicknesses. The different layers originated from a depth of 0-1, 1-2, 2-3, 3-4, 4-6, 6-10, 10-15, 15-20, and 20-25 cm. The soil weight per layer was determined and the soil was wetly sieved to pass a sieve of 5 mm. Organic matter was separated from the soil using a saturated calcium chloride solution. Seeds that resisted gentle pressure were classified as apparently viable (Roberts & Ricketts 1979) and removed from the organic matter. The apparently viable seeds were laid out to germinate at an assumed optimal germination temperature of 15/25 °C with 12 h of light and 12 h of darkness. Seeds which did not germinate were tested for viability using tetrazolium (Moore 1973). In this way, the number of viable seeds per soil layer at the end of the growing season was determined for each separate cylinder. The number of viable seeds per layer was expressed as number of seeds per kg of soil. The binomially distributed parameter number of viable seeds per kg of soil was analysed with a stepwise multiple linear regression (Payne *et al.* 1987). It was assumed that seeds initially were homogeneously distributed over the soil profile.

Experiment 2

The fate of triazine susceptible and resistant seeds of *S. nigrum* outside the growing season was determined in 1996. In September 1995, ripe berries were harvested from *S. nigrum* plants grown in an experimental field under similar environmental conditions with no inter- or intra-specific competition. Populations originated from two different maize fields, Achterberg (51°59' N, 5°35' E) and Zelhem (51°59' N, 6°18' E) with both biotypes present in each field. Triazine resistance of plants was confirmed

Table 6.1. The mean seed weights of different seed weight fractions of two biotypes from two populations. Standard errors of the means are in parentheses.

Population	Biotype	Fraction	Seed weight (10^{-6} g)
Achterberg	Susceptible	I	785 (7.6)
		II	805 (6.5)
		III	880 (6.7)
	Resistant	I	728 (6.7)
		II	752 (6.8)
		III	794 (6.3)
Zelhem	Susceptible	I	677 (8.7)
		II	759 (8.0)
		III	820 (7.7)
	Resistant	I	746 (7.1)
		II	785 (7.0)
		III	800 (6.0)

using measurements of quantum yield as previously described.

Berries were collected from 10 to 19 plants per biotype. Seeds were washed, sieved and dried at room temperature. Dry seeds from each biotype and population were divided into three batches with equal numbers of seeds and different seed weights that were attained by separation with air, using a South Dakota blower (model 757, Seedburo Equipment Co., Chicago, USA). Individual seed weights per batch and their standard errors were determined by weighing 200 single seeds (Table 6.1). Experimental set-up consisted of a randomized design with the treatments population, biotype, seed weight, exhumation date and germination temperature in two replicates.

The nylon bags, filled with a mixture of sandy soil and 100 seeds of a specific treatment were buried in the field at a depth of 5 cm in December 1995. Bags, containing seeds from different treatments, were dug up at monthly intervals on 1 February, 1 March, 1 April and 1 May 1996 and laid out to germinate at different temperatures (for details see Kremer & Lotz 1998a. Chapter 2). Besides the germination fraction, the dormancy fraction, and the mortality fraction of the seeds was also determined. The number of dead seeds was binomially distributed and analysed with a stepwise multiple linear regression. Mortality data of the exhumation date 1 February were ignored in the analysis because of the illogical high mortality fraction which would have resulted in a decreased seed mortality in time.

Results

Experiment 1

The emergence patterns of triazine susceptible and resistant seedlings in the 1995 season are presented in Fig. 6.1. Total number of seedlings did not differ significantly between biotypes, and represented 8.3 and 7.5% of the total number of buried seeds in the 25 cm deep soil column for the susceptible and resistant biotype, respectively.

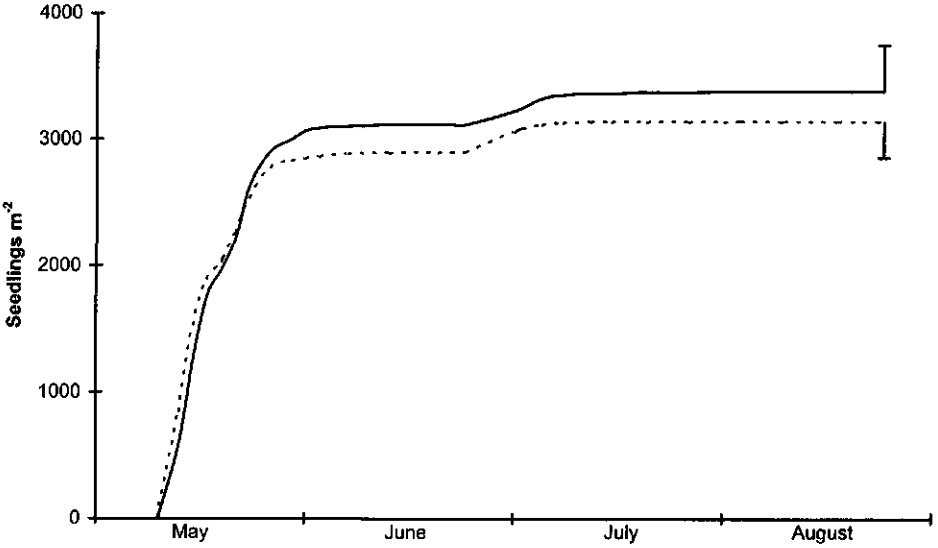

Fig. 6.1 The number of emerged seedlings of triazine susceptible (———) and resistant (– – – –) biotypes from the Achterberg population during the 1995 experiment. Vertical bars indicate standard errors.

The fraction of surviving seeds from both biotypes of the Achterberg population at the end of the growing season at different depths in the soil is given in Fig. 6.2, while the accompanying average daily soil temperatures at different depths are shown in Fig. 6.3. More seeds survived with increasing soil depth, the susceptible seeds being more persistent than the resistant seeds. There was a significant interaction between depth and biotype ($P < 0.001$). The maximum difference in fraction of viable seeds between biotypes was 0.24 and found in the layers between 6 and 15 cm deep. Between 20 and 25 cm depth, the difference in survival fraction was 0.16. The fate of all buried viable seeds in the soil is illustrated in Fig. 6.4 with the assumption that the seedlings had a maximal emergence depth of 5 cm (Kremer & Lotz 1998b). The viable seeds were divided into germinable and non-germinable ones at a temperature of 15/25 °C because no conclusive evidence of the dormancy state of the seeds can be drawn from germination at a single temperature regime (Vleeshouwers *et al.* 1995). Lost seeds not recovered from the soil were assumed to have decayed. Accumulated over the whole tilth of 25 cm, the mortality fraction of susceptible seeds was 0.52 and that of resistant seeds 0.72.

Experiment 2

In 1996, the mortality fraction of triazine susceptible and resistant *S. nigrum* seeds from different populations with different seed weights was determined during the

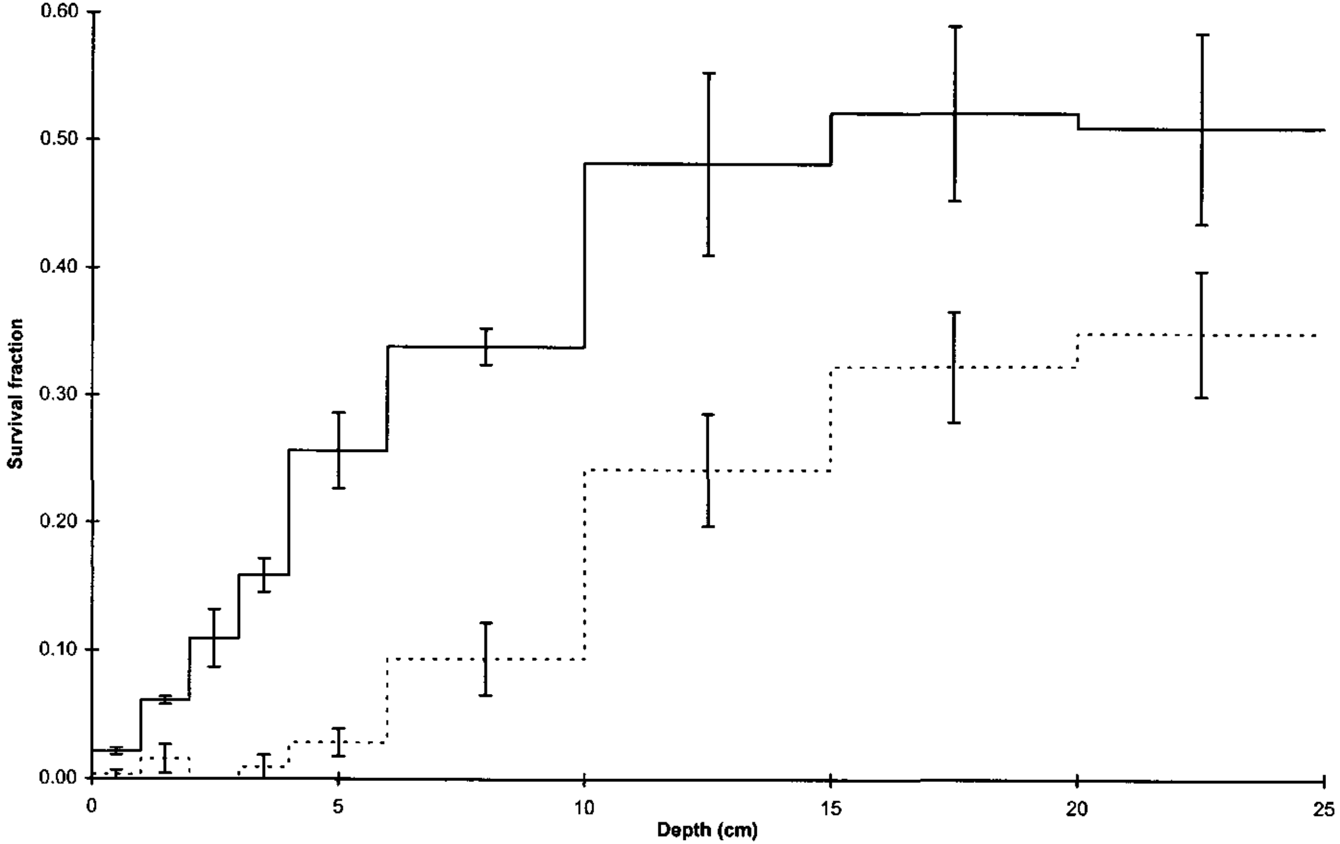

Fig. 6.2 The survival fraction of triazine susceptible (———) and resistant (- - - -) seeds from the Achterberg population at different depths, determined in October 1995 at the end of the growing season. Vertical bars indicate two times the standard error.

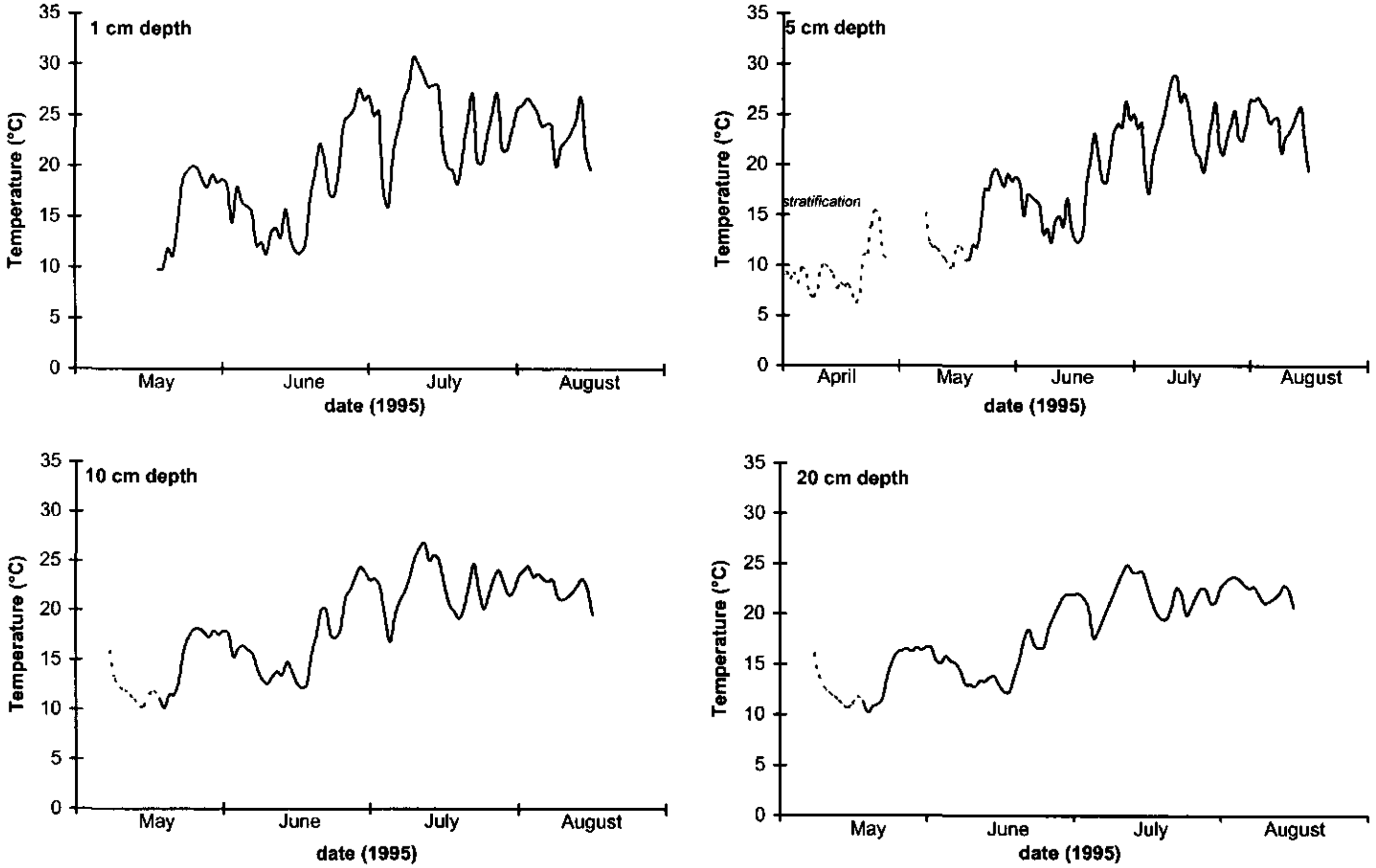

Fig. 6.3 Average daily soil temperatures at selected depths during the 1995 experiment. At the start of the season, soil temperatures were retrieved from a nearby local weather station in Wageningen (broken lines).

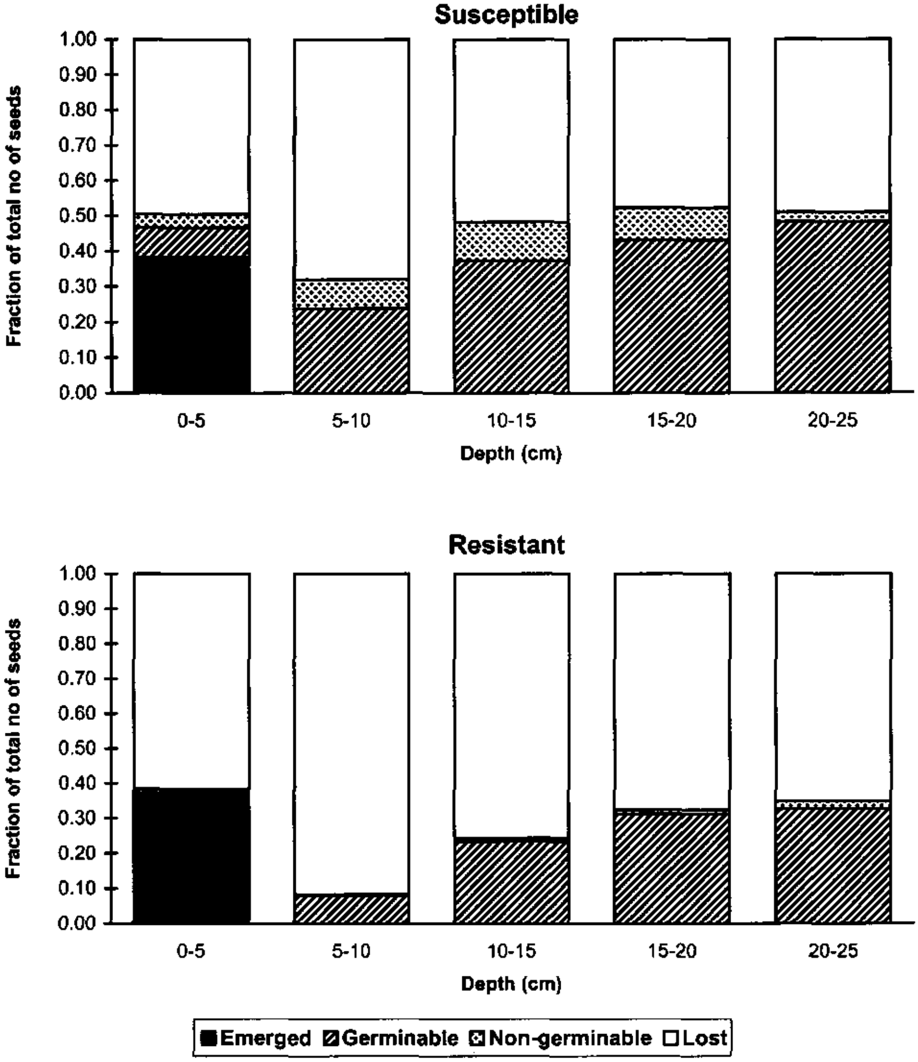

Fig. 6.4 The fate of seeds in the soil expressed as fraction of the initial number of seeds per soil layer.

winter and early spring. The analysis showed a significant effect of exhumation date on the mortality fraction ($P < 0.001$) which increased from 0.009 on 1 March through 0.014 on 1 April to 0.020 on 1 May. The analysis did not include the mortality fraction of exhumation date 1 (1 February) which was unrealistically high, probably due to exhumation from frozen soil combined with a high defrosting rate of the seeds (Table 6.2, Fig. 6.5). The mortality fraction was also significantly affected by population ($P < 0.001$) and biotype ($P = 0.05$; Table 6.2). At the end of the experiment, seeds from the

Table 6.2. Mortality fraction of seeds from susceptible and resistant biotypes from two populations at different exhumation dates buried in December of the preceding year. Standard errors of the means are in parentheses.

Population	Achterberg		Zelhem	
Biotype	Susceptible	Resistant	Susceptible	Resistant
1 February	*0.030 (0.0112)*	*0.015 (0.0044)*	*0.020 (0.0038)*	*0.012 (0.0026)*
1 March	0.007 (0.0020)	0.007 (0.0029)	0.013 (0.0016)	0.010 (0.0017)
1 April	0.005 (0.0015)	0.011 (0.0049)	0.020 (0.0021)	0.019 (0.0064)
1 May	0.012 (0.0027)	0.023 (0.0029)	0.020 (0.0031)	0.027 (0.0035)

Zelhem population had a higher mortality than those of the Achterberg population and seeds of the resistant biotype had a higher mortality than those of the susceptible biotype. The average mortality fraction decreased with increasing seed weight ($P < 0.001$; Fig. 6.6) and this effect was independent from population or biotype.

Discussion

The highest weed mortality occurs during the seed stage of the life cycle (Yenish *et al.* 1992). Manipulation of the seed bank may therefore help to control herbicide resistant weed populations. Differences in longevity, dormancy, and germination characteristics of susceptible and resistant seeds in the soil may be more determinative for differences in fitness between biotypes than differences in growth and reproduction.

In the experiments with triazine susceptible and resistant *S. nigrum* seeds, the mortality fraction during the winter and early spring was very low (< 0.04) and slightly

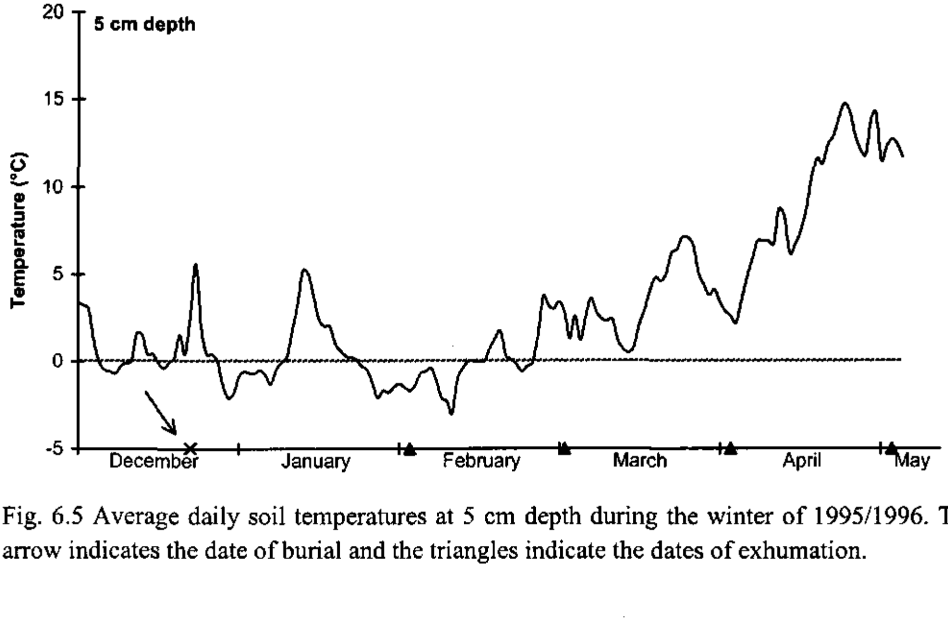

Fig. 6.5 Average daily soil temperatures at 5 cm depth during the winter of 1995/1996. The arrow indicates the date of burial and the triangles indicate the dates of exhumation.

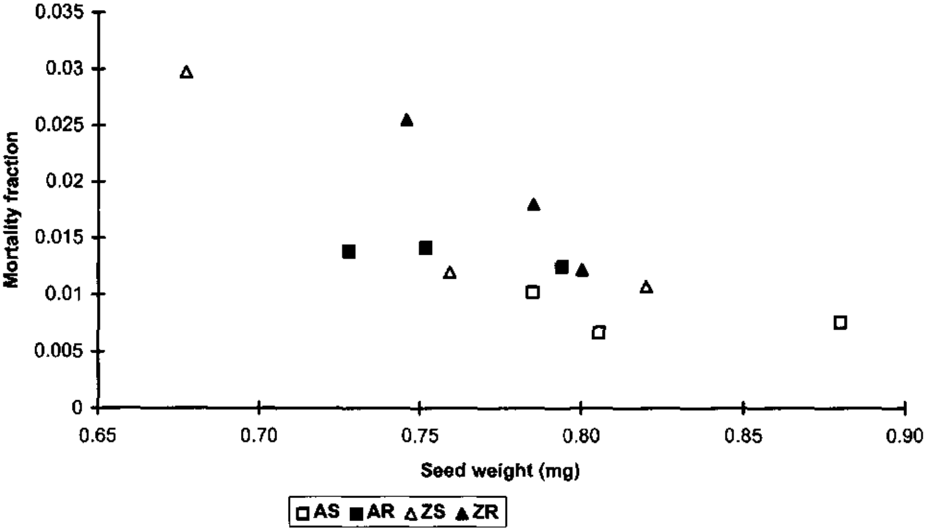

Fig. 6.6 Mortality fraction of seeds from triazine susceptible (AS) and resistant (AR) biotypes of the Achterberg population and from triazine susceptible (ZS) and resistant (ZR) biotypes of the Zelhem population as a function of individual seed weight.

higher for the resistant biotype than for the susceptible biotype. In summer, however, differences in seed survival between susceptible and resistant biotypes were much larger. At the end of the growing season the viable fraction of resistant seeds in the tilth (25 cm deep) was only 0.28 compared to a viable fraction of 0.48 for the susceptible seeds. The higher germination fraction at 15/25 °C of the viable resistant seeds compared with susceptible seeds is consistent with data on differences in dormancy and germination of triazine susceptible and resistant *S. nigrum* seeds in spring (Kremer & Lotz 1998a).

Seeds are lost from the soil by recruitment of seedlings from the soil seed bank. The number of weed seedlings varies from year to year and depends upon the environmental conditions in the soil, the density and viability of the seed bank, the depth of seed burial, and germination characteristics of the species. In experiment 1, the number of emerged seedlings represented 8.3 % and 7.5 % of the susceptible and resistant seed bank, respectively. These percentages are close to the range of 3 % to 6 % emergence from weed seed banks reported by Buhler *et al.* (1997). Differences in number of seedlings between susceptible and resistant biotypes of *S. nigrum* could be expected at low soil temperatures as a result of differences in minimum germination temperature requirements (Kremer & Lotz 1998a). However, since the soil temperature in April 1995 already exceeded minimum germination temperatures of both biotypes, no significant difference in number of seedlings between biotypes was observed.

Seeds are also lost from the soil by predation and decay. Predation mainly takes place on seeds that remain on the soil surface. In agricultural systems, this seems to be of minor importance due to intensive soil disturbance, seed burial by tillage, and lack of habitats for predators (Buhler *et al.* 1997). Decay of the seeds in the soil by soil micro-organisms contributes to the depletion of the seed bank but it is unclear to what extent (Kremer 1993). Decay of triazine susceptible and resistant *S. nigrum* seeds outside the growing season at low temperatures, seemed to be insignificant as was shown by the very low mortality fractions (Table 6.2). In the growing season, soil temperatures were higher and soil life became more active, probably resulting in an increased mortality of seeds due to decay.

Seeds in the soil can also germinate without emerging. At greater depths, germination and dormancy depend among other things on light, temperature ranges and fluctuations, and oxygen concentrations (Dyer 1995). Emergence of germinated seeds mainly depends on soil characteristics and seed weight (Wilson 1988). Froud Williams *et al.* (1984) reported for most arable weed species a critical emergence depth of 5 cm. This was also observed for *S. nigrum* by Kremer and Lotz (1998b, Chapter 3) who found, under controlled conditions, a slightly higher emergence fraction of germinated seeds of the resistant biotype compared to that of the susceptible biotype. Vleeshouwers (1997) developed a physiological model to describe the effects of temperature, soil penetration resistance, burial depth, and seed weight on pre-emergence growth of seedlings after germination. The emergence fraction was unaffected by temperature but decreased with increasing soil penetration resistance, burial depth, and lower seed weights. The small differences in seed weight between the susceptible and resistant biotype observed in this experiment, could, however, not explain the large differences in seed mortality as a possible consequence of unsuccessful emergence of the seedlings.

The average daily soil temperature and its fluctuations decreased at greater depths (Fig. 6.3). Lower minimum germination temperatures of resistant seeds (Kremer & Lotz 1998a, Chapter 2) could imply that at greater depths relatively more resistant seeds than susceptible seeds germinated without emerging. At 10 cm depth, the soil temperature did not exceed the assumed minimum germination temperature of the susceptible biotype of 17 °C (Keeley & Thullen 1983) until the end of June possibly resulting in fatal germination of mainly resistant seeds. This assumption seems to be supported by the fact that differences in survival fraction between the susceptible and resistant biotype were largest between 6 and 15 cm deep. At greater depths, other environmental conditions probably prevented seeds from both biotypes from germination.

The longevity of seeds in the soil seed bank increases with an increasing depth of burial and a decreased intensity of soil disturbance (Schweizer & Zimdahl 1984). Watson *et al.* (1987) studied the longevity of triazine susceptible and resistant achenes of *Senecio vulgaris* in a management system with one soil cultivation in spring. They distinguished a superficial seed bank with a higher mortality rate of resistant seeds and a lower seed bank with a higher mortality rate of susceptible seeds. In experiment 1 of the present investigation, the mortality fraction of both biotypes at greater depths is very high with a higher mortality fraction of the resistant seeds. In the range of 10-25 cm, the mortality fraction of the susceptible seeds is 0.50 and that of resistant seeds 0.70. Besides a difference in seed weight (Fig. 6.6), a large part of the difference in seed mortality between biotypes at greater depths involved other seed characteristics which are yet unclear. Touraud *et al.* (1987) found that triazine resistant seeds of *Poa annua* had a lower abscisic acid content than susceptible seeds where abscisic acid might be one of the factors inhibiting germination (Kigel & Galili 1995). Unfortunately, hormonal composition of seeds from susceptible and resistant biotypes of other species have never been determined, not confirming the potential hormonal effect on seed longevity.

The survival of *S. nigrum* seeds in the soil has been studied before. Already in 1946, it was reported that after 39 years without soil disturbance viable seeds of *S. nigrum* were still found in the Duvel burial experiment (Toole & Brown 1946). Experiments with *S. nigrum* seeds in soil that was periodically disturbed showed that after 5 years 11% of the seeds were still present and viable (Roberts & Lockett 1978) and that 2.1% of the original seeds emerged as seedlings (Roberts 1986). The highest seed mortality rate is in general observed in management systems which include spring ploughing (Semb Tørresen 1998).

In experiment 1, which included an intensive soil disturbance at the start of the growing season, the seed survival over a year is much higher for the susceptible biotype than for the resistant biotype. Differences in longevity between susceptible and

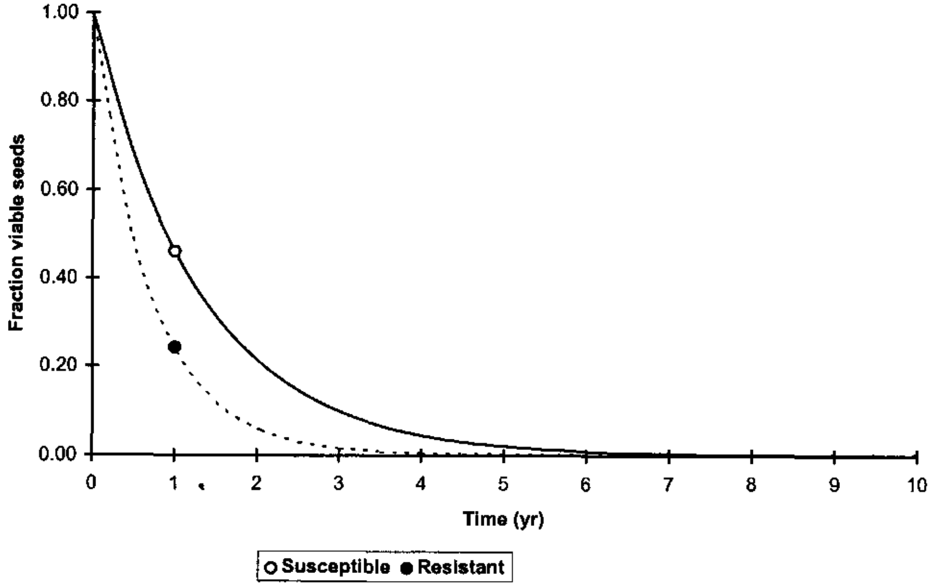

Fig. 6.7 The exponential decay curve of triazine susceptible and resistant seeds of *S. nigrum* based on the viable fraction found after one year.

resistant seeds in this single experiment might be translated to other years, although the extent of the differences might vary between years. The higher survival of susceptible seeds will result in a continued recruitment of susceptible individuals from the persistent seed bank and delay the development of herbicide resistant weed populations (Powles *et al.* 1997). A reduced longevity of resistant seeds might also be used to specifically exhaust the resistant seed bank. Despite the high variation in seed longevity between species and cropping systems, in general, a negative exponential decline of the number of viable seeds in the soil is assumed when seed input is prevented (Roberts & Feast 1972, Mohler 1993, Burnside *et al.* 1996). With this assumption, the time needed for exhaustion of the susceptible and resistant seed bank, based on the viability loss in the first year, is shown in Fig. 6.7. A reduction of the seed bank to less than 1 % of the original seed bank should then take 6 years for the susceptible biotype and only 3 years for the resistant biotype when seed input is prevented. The results of this single experiment show that prevention of seed input in the seed bank by crop rotation and reduction of seed production might be an useful tool in the management of triazine resistant weed populations. In a cropping system with continuous maize, a reduction of the seed production of both biotypes should be reached by increasing competitive ability of the crop combined with other mechanical and chemical weed control options.

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Chapter 7

Population dynamics of triazine susceptible and resistant *Solanum nigrum* in maize: a simulation analysis

Abstract

A population dynamics model was developed to quantify fitness differences between triazine susceptible and resistant *Solanum nigrum* in a maize agro-ecosystem. The model was parameterized using experimental data from studies on different life history processes such as germination, emergence, growth, seed production, and seed persistence in the soil. Fitness of the resistant biotype was lower than that of the susceptible biotype, mainly because of differences in seed characteristics between biotypes. The effect of different weed control measures and their selection pressure on the population dynamics of triazine susceptible and resistant *S. nigrum* in maize was studied. It was shown that triazine resistance management strategies based on fitness differences between susceptible and resistant biotypes should be aimed at depletion of the seed bank of the resistant weed population.

Key-words: black nightshade, fitness, population dynamics, triazine resistance management.

Introduction

The widespread and intensive use of herbicides with a high selection pressure in different cropping systems has resulted in the development of herbicide resistant weed populations. The most frequently reported cases concern concern triazine resistance in maize cropping systems or orchards (Heap 1997). The failure of chemical weed control measures in these systems urges the development of alternative weed management strategies. Long-term management strategies must be based on an understanding of the biological, ecological, and economic processes that drive the cropping system (Roush *et al.* 1990).

To study the development rate of herbicide resistance and its management, theoretical models have been developed by Gressel and Segel (1990) and by Maxwell *et al.* (1990). According to Gressel and Segel (1990), the success of management of resistant weeds is mainly driven by the selection pressure of the herbicide, the seed bank dynamics of both biotypes, the fitness of the resistant biotype compared to that of the susceptible biotype, and the herbicide rotation system. In their model, the often reported differences in fitness between susceptible and resistant biotypes in the absence of the selecting herbicide were described by one parameter only. This single fitness parameter was assumed to describe the accumulated relative fitness of resistant biotypes in competition with susceptible biotypes during germination, establishment, growth, pollination, seed production, and survival in absence of the selecting herbicide.Maxwell *et al.* (1990) introduced gene flow in their model and recognized the importance of different life history stages and their accumulated effect on overall fitness of susceptible and resistant biotypes. The different submodels described seed bank dynamics, seed immigration, seedling survival, mature plant survival, pollen immigration, pollen production, inheritance, and seed yield. In their theoretical model, the different variables were arbitrarily chosen and varied in the different simulation runs. The model simulated the evolution, spread, and dynamics of susceptible and resistant biotypes based on biological processes without experimental backup.

The models developed so far were not tested or parameterized with experimental data. Both Gressel and Segel (1990) and Maxwell *et al.* (1990) used hypothetical parameter values (guestimates) in their simulations which were varied to study their effect on resistance development. Field ecological data are needed to test the validity and predictive value of the models proposed (Warwick 1991). Mortimer *et al.* (1992) used the results of an experiment on the effects of competition and herbicide rate on the fitness of chlorotoluron susceptible and resistant *Alopecurus myosoroides* in the simulation of different management regimes while the other parameters were guestimates. Zanin and Lucchin (1990) used data on the fitness of triazine susceptible and resistant *Solanum nigrum* from a replacement series experiment in the evaluation of the effect of selection pressure and crop rotation on the resistance frequency in the population. In both cases, the fitness was characterized by seed production per plant only. The other parameters used in the simulations such as allele frequency and selection pressure (Mortimer *et al.* 1992; Zanin and Lucchin 1990) and seed longevity (Mortimer *et al.* 1992) were based on guestimates which were varied for each biotype. The use of fitness based on seed production of susceptible and resistant biotypes in competition probably overestimated the fitness cost of the resistant biotype in field situations at lower plant densities.

Models provide a logical structure to conceptualize and organize biological processes, to explore interactions among processes, and to simulate responses of weed populations to management decisions (Roush *et al.* 1990). The presence of the selecting herbicide strongly increases the relative fitness of the resistant biotype. However, when herbicide selection is removed, differences in population dynamics are determined by differences in all life history processes that contribute to the fitness of the susceptible and resistant biotypes (Maxwell *et al.* 1990). The variables measured to estimate fitness of susceptible and resistant biotypes must reflect the most important life history processes that most directly result in changes in resistance gene frequencies in the weed population (Jasieniuk *et al.* 1996). Mathematical simulation using realistic parameters derived from field studies will give the best possible prediction of the outcome of herbicide management regimes (Putwain & Mortimer 1989). Therefore, field data on germination, dormancy, emergence, survival, and seed production of susceptible and resistant biotypes should be used for parameterization and evaluation of a simulation model to approximate herbicide resistance dynamics in the field. Once the herbicide resistance dynamics in the field are known, management strategies to control resistant weeds in a specific cropping system can be developed.

In this study, a simulation model for the population dynamics of triazine susceptible and resistant *S. nigrum* in the agro-ecosystem maize was developed. Data on the biology of susceptible and resistant biotypes of *S. nigrum* were used for parameterization of the population dynamics model. The model was used to estimate the fitness of the susceptible and the resistant biotype in a maize crop in the absence of the selecting herbicide. The effect of different management tactics on the development of triazine susceptible and resistant *S. nigrum* populations in maize was evaluated with the model.

Material and methods

System description. The model simulates the population dynamics of triazine susceptible and resistant biotypes of *S. nigrum* in an agro-ecosystem with continuous cropping of maize. The genetics of *S. nigrum* are relatively simple as the species is a self-pollinator and outcrossing is excluded. Triazine resistance, which is located on the chloroplast genome, is maternally inherited. Maize is continuously cropped at a plant density of ± 9.5 pl m^{-2} and the field is ploughed just before sowing of the crop. *S. nigrum* and other weeds are controlled in such a way that interactions between susceptible and resistant biotypes and other weeds are prevented. The crop is harvested mid-October and a large part of the *S. nigrum* population, including berries, is removed from the field with the crop.

General structure. The model encompasses behaviour of both biotypes in the different life history processes in the absence of triazine herbicides, based on experimental data. The distinguished life history stages are seeds in the seed bank, germinated seeds, seedlings, mature plants, produced seeds, and seeds removed with the harvest (Fig. 7.1). The population is represented by number of individuals per m^2, either seeds or plants and the time step of integration is one year. Biological data on plant growth and seed production are based on freely growing *S. nigrum* plants in a maize crop based on the assumption that in practice, high *S. nigrum* densities are not tolerated. The effect of weed control measures in the model was expressed by a fraction mortality of germinated seeds and/or a fraction mortality of plants (Fig. 7.1).

Table 7.1. The mortality fraction of non-germinated triazine susceptible and resistant seeds of *S. nigrum* in different layers of the seed bank during the year (Chapter 6).

Depth (cm)	Mortality fraction	
	Susceptible	Resistant
0-5	0.65	1.00
5-10	0.68	0.95
10-15	0.56	0.79
15-20	0.51	0.71
20-25	0.50	0.69

State variables and processes

Seed bank. The most common depth of tilth in a maize cropping system is 25 cm. The tilth was divided into 5 layers of 5 cm depth each where each layer has its own mortality based on data derived from a field experiment (Table 7.1). Shortly before sowing of the crop, the soil is ploughed and seeds from the different soil layers are redistributed. Redistribution takes place according to a matrix for seed movement in the soil reported by Cousens and Moss (1990) which was adapted for a tilth with 5 instead of 4 layers by interpolation:

$$\begin{array}{llccccc} & & & & \textit{Soil layer before} & & \\ & & \textit{0-5} & \textit{5-10} & \textit{10-15} & \textit{15-20} & \textit{20-25} \\ & \textit{0-5} & 0.00 & 0.14 & 0.24 & 0.28 & 0.26 \\ & \textit{5-10} & 0.08 & 0.20 & 0.24 & 0.20 & 0.07 \\ \textit{Soil layer after} & \textit{10-15} & 0.37 & 0.29 & 0.21 & 0.13 & 0.06 \\ & \textit{15-20} & 0.40 & 0.26 & 0.17 & 0.15 & 0.18 \\ & \textit{20-25} & 0.14 & 0.10 & 0.14 & 0.24 & 0.42 \end{array} \qquad \text{(Eq. 7.1)}$$

The redistribution of seeds from the upper soil layer according to Cousens and Moss (1990) was fitted with the function described by Staricka *et al.* (1990), also successfully used by Yenish *et al.* (1996):

$$VS_i = \frac{1}{[1+\exp(b_0)\times\exp(b_1.Z_i)} \qquad \text{(Eq. 7.2)}$$

In this function, the cumulative fractional distribution of seeds to the *i*th depth (VS_i) is based on the midpoint depth of the sample (Z_i) and two shape parameters (b_0 and b_1). The redistribution of the upper soil layer was fitted with this function and the number of soil layers to which seeds moved was changed from 4 to 5. Redistribution of seeds from the other soil layers as described by Cousens and Moss (1990) was fitted with a polynomial for each individual layer and subsequently divided in movement to 5 layers instead of 4 layers. The matrix which was derived resulted in a homogeneous frequency distribution of seeds after simulation for 20 years without seed input into and output from the soil.

Germination. Seeds can only germinate in the upper 5 cm soil layer and germination probability is constant for each year. Germination as a function of time is described with a Gompertz curve. Germination starts at the day the crop is sown and is divided into 5 cohorts with different germination probabilities (Table 7.2). Although the dormancy of susceptible and resistant seeds differed during the rest of the year (Kremer & Lotz 1998b, Chapter 2), the observed germination probabilities in spring at optimum soil temperatures were similar and assumed to be equal at a value of 0.85. The lower minimum germination temperature requirement of resistant seeds (Kremer

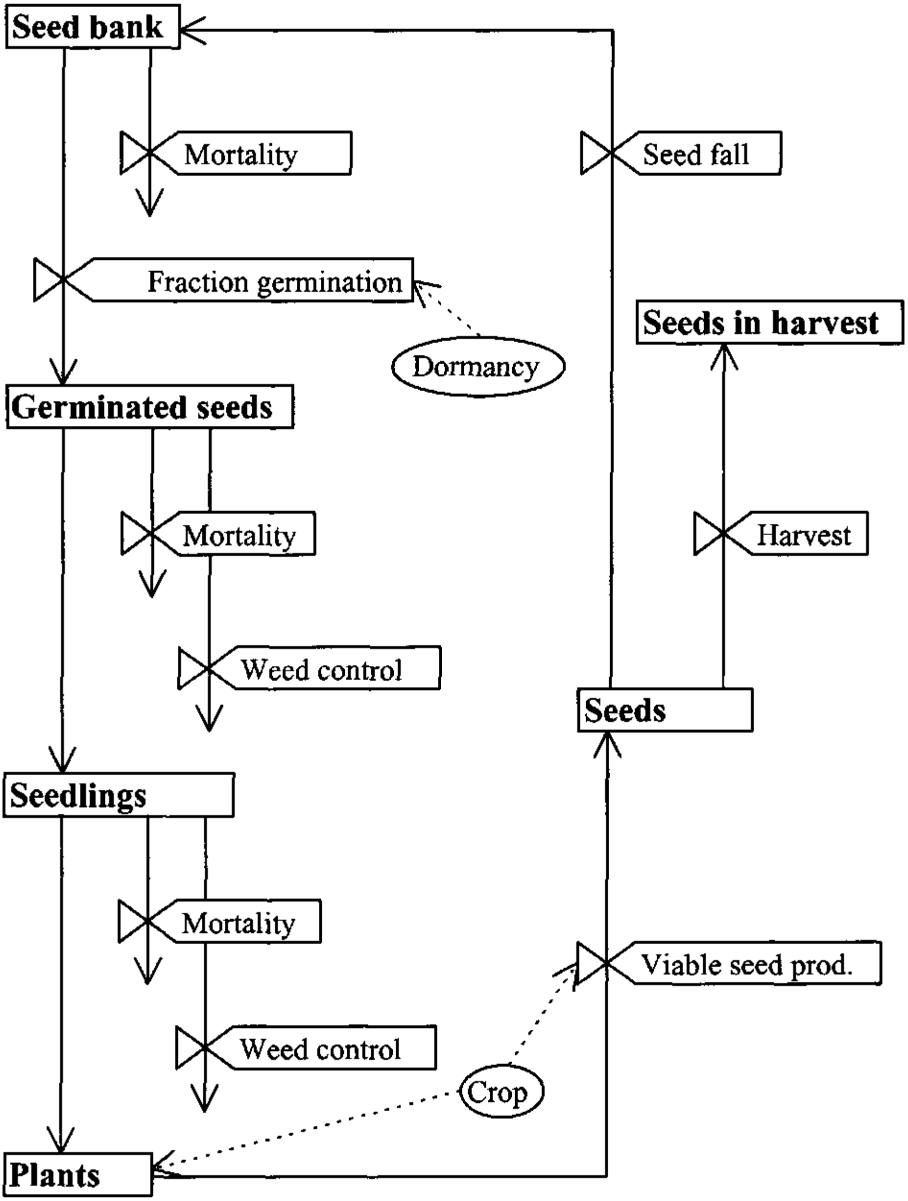

Fig. 7.1 A representation of the population dynamics model that follows the different life history stages of triazine susceptible and resistant biotypes of *S. nigrum* in the agro-ecosystem with continuous cropping of maize.

Table 7.2. The germination probabilities of each cohort, which were set equal for the susceptible and resistant biotypes.

Cohort	Germination probability
1	0.085
2	0.238
3	0.332
4	0.153
5	0.043
Total	0.85

& Lotz 1998b, Chapter 2) will result, depending on the soil temperature at the time of sowing, in earlier germination of resistant seeds in some years. The earlier germination of resistant seeds, which is assumed to happen once in three years (pers. obs.), was randomized over the simulation period. In that case, the first germination cohort of resistant seeds was completely killed by the seedbed preparations and the subsequent cohorts germinated one week earlier.

Emergence. The emergence probability of germinated seeds differed for the susceptible and resistant biotype. A Gompertz curve was fitted through the emergence data of Kremer and Lotz (1998a, Chapter 3) to calculate the probability of successful emergence of germinated seeds from the upper soil layer of 5 cm. The fraction natural mortality of germinated seeds (seeds which did not successfully emerge) in the upper soil layer was 0.43 for susceptible seeds and 0.36 for resistant seeds (Table 7.3) and assumed to be the same for all cohorts. Besides natural mortality due to unsuccessful emergence, mortality of germinated seeds can also occur from weed control measures.

Biomass and seed production. During the seedling phase in a maize crop, plants face a natural mortality which is assumed to be the same for each cohort and equal for both biotypes and estimated to be 0.14 based on field observations (unpubl. results Kremer). Weed control measures can result in mortality which can be varied for each cohort and each biotype. The final seed production of freely grown plants in a maize crop was estimated from an irrigated growth experiment of both biotypes in a maize crop (Table 7.3; Kremer & Kropff 1998a, Chapter 5). A delayed emergence date of the weed compared to that of the maize crop showed an exponential decline of the seed production because of increased competition (Kremer & Kropff 1998a, Chapter 5). Therefore, each germination cohort had its own level of seed production based on the time of emergence (Table 7.2). The time of emergence for each cohort was 6 days after germination, based on the average t_{50} for emergence of germinated seeds from the upper soil layer of 5 cm (Kremer & Lotz 1998a, Chapter 3).

Seed input in the seed bank. A fraction of the total number of seeds produced is added to the upper 5 cm of the seed bank while the rest is removed by harvest. Because of the sympodial flowering and ripening of berries of *S. nigrum*, this fraction depends on the germination cohort and is estimated by the number of berries which have fallen during the growing season. Equal emergence dates of *S. nigrum* and maize crop

Table 7.3. Summary of the parameter estimates for the population dynamics model of triazine susceptible and resistant *S. nigrum*.

Description	Biotype	
	Susceptible	Resistant
Germination probability (fraction)	0.85	0.85
Natural mortality germinated seeds (fraction)	0.43	0.36
Natural mortality plants (fraction)	0.14	0.14
Final seed production (seeds/plant)	28992	19152
Fraction seed fall (fraction)	0.21	0.13
Seed viability (fraction)	0.95	0.95

resulted in a fraction seed fall of 0.21 for the susceptible biotype and 0.13 for the resistant biotype. As *S. nigrum* plants emerge later, the fraction seed fall decreases. Berries which are still attached to the plant at harvest time are assumed to have being removed from the field with the harvest.

Weed control. Besides physiological and ecological characteristics of both biotypes, the most important other characteristic governing the development and management of herbicide resistance is selection pressure (Gressel & Segel 1990). The selection pressure of a control measure is defined as the fraction survival of resistant plants divided by the fraction survival of susceptible plants. Non-selecting weed control measures e.g. soil tillage have a selection pressure 1. The selection pressure is modelled by introducing a parameter for weed control per cohort for each biotype separately.

The effect of different weed control measures is expressed by a mortality fraction of germinated seeds and/or plants for each cohort which are derived from literature (Zanin & Lucchin 1990, Van der Weide *et al.* 1995). Mortality can be the result of soil tillage before or after emergence of the maize and by weed control measures such as harrowing, hoeing, band spraying or full field application of a contact or a soil herbicide. Whenever a contact herbicide is applied, the resulting mortality is restricted to the first four cohorts. One of the assumptions in the model is that the last cohort of *S. nigrum* emerges after the last possible weed control measure in the maize crop and can only be controlled by a soil applied herbicide.

Model application. In the simulations, realistic parameters derived from the different experiments were used (Table 7.3). The effect of different weed control measures was studied by varying the artificial mortality fractions of germinated seeds and plants within realistic boundaries for a period of 20 years. Optimal strategies to reduce the resistant population and at least stabilize the susceptible population were determined. The potential management options in the field are discussed.

Results and discussion

The initial seed numbers in the simulations consisted of 100 seeds from each biotype in every soil layer. In total, the simulation started with 500 susceptible seeds and 500 resistant seeds, resulting in a resistance fraction of 0.50. In the first simulations the parameter value set for the susceptible biotype was taken. Weed control was adjusted in such a way that the population was stable over time (Table 7.4). However, large fluctuations in seed bank size in the first few years of the simulation were observed before stabilizing. The seed input into the seed bank through seed production in the model was added to the seeds in the upper 5 cm soil layer. As indicated by the seed redistribution matrix (Eq. 7.1), seeds from the top soil layer are completely moved to deeper soil layers by ploughing, which means they do not contribute to the upper soil layer from which seeds can successfully emerge the next year. Only from the second year after seed production on, produced seeds can contribute to the seedling population. After 6-8 years of simulation, the seed numbers in the different soil layers stabilize.

Model simulations showed that without control of germinated seeds, which represents e.g. a soil applied herbicide or harrowing, the kill rate of a contact herbicide had to exceed 0.98 to control the susceptible biotype which is exceptionally high. Studies on the control of *Galium aparine* in wheat showed that the smallest kill rate preventing population increase, called critical kill rate, was 0.95 or higher (Wilson & Wright 1991, Wallinga 1998). Since a kill rate of 0.98 is not very realistic, a successful management strategy to control *S. nigrum* in maize should include the control of germinated seeds. The kill rates of control measures to control germinated seeds such as harrowing, hoeing or a non-selecting soil herbicide vary in practice between 0.7 and 0.8 (Zanin & Lucchin 1990, Van der Weide *et al.* 1995). A kill rate of the germinated seeds of 0.7 or 0.8 still required a kill rate of the plants of 0.96 or 0.93, respectively, to control the susceptible biotype.

Using the parameters of the susceptible and resistant population, control with non-selecting weed control actions (selection pressure 1) showed a strong decrease in the resistant population, indicating that it was much more difficult to control the susceptible biotype than the resistant biotype (Fig. 7.2). Control of the susceptible biotype aimed to keep the population size at a constant level, resulted in a reduction of the resistant biotype, which ultimately disappeared after 11 years in absence of the selecting herbicide. This meant that, when the selection pressure was 1, the resistant biotype disappeared eventually because of its reduced fitness. With the simulation

Table 7.4. The kill rates of germinated seeds and plants from different cohorts of the susceptible biotype used to stabilize the population size.

Cohort	Kill rate	
	Germinated seeds	Plants
1	0.7	0.95
2	0.7	0.95
3	0.7	0.95
4	0.7	0.95
5	0.7	0.95

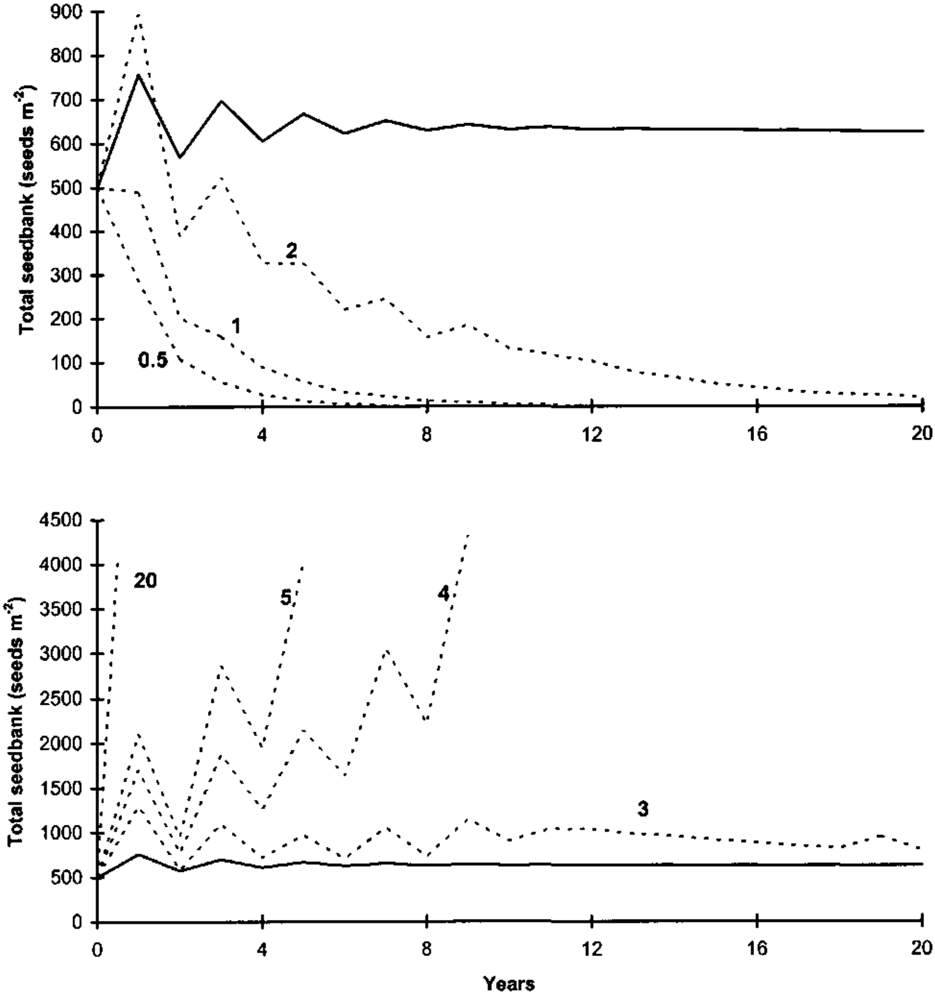

Fig. 7.2 Calculated population dynamics of triazine susceptible (——) and resistant (- - -) biotypes of *S. nigrum* in maize expressed as the total seed bank over 20 years. The basic weed control parameters were set in such a way that the susceptible population size is kept constant and subsequently different selection pressures (number) were applied. The population increase ends when the plant density exceeded the maximum level of 5 plants per m^2, set in the model.

model, fitness differences between the susceptible and the resistant biotype are quantified by the integration of different life history processes. Starting with equal seed bank sizes of both biotypes, with kill rates that keep the susceptible population size stable, the population size of the susceptible and resistant biotype after one year was compared to quantify the relative fitness. The relative fitness of the resistant biotype in comparison with that of the susceptible biotype valued 0.65. Despite the strongly reduced fitness of the resistant biotype, it still takes 11 years in the absence of the selecting herbicide before the biotype disappears from the population.

The population dynamics of the susceptible and resistant biotype diverged as soon as the selection pressure changed. In this study, the kill rate of the germinated seeds was held constant at a realistic value of 0.7. Selection pressure was varied by changing the kill rate of resistant plants while keeping the kill rate of susceptible plants constant at 0.95. A selection pressure smaller than 1 meant that the herbicide controlled triazine resistant biotypes better than susceptible biotypes. If twice as many susceptible individuals survived the weed control measure than resistant individuals (selection pressure 0.5), the resistant biotype would disappear within 6 years (Fig. 7.2). Even with an increase of the selection pressure to a value 2 (twice as many resistant as susceptible individuals survive) the resistant biotype disappeared because of its reduced fitness. As soon as three times as many resistant individuals survived the weed control measure as susceptible individuals, the resistant population size remained stable. Further increasing the selection pressure resulted in a very steep increase of the resistant population, soon limited by the maximum plant density of 5 plants m^{-2} set in the model. A selection pressure of a triazine herbicide, which lies in the range 20-50, results in an explosion of a resistant population when use of the selecting herbicide would be continued (Fig. 7.2) as is also found in practice (pers. obs.). The effective management of herbicide resistance in weeds depends on reduction of the selection pressure, which involves reducing the frequency and amount of herbicide applied and an increasing reliance on integrated management practices (Holt *et al.* 1993).

Sensitivity analysis

Sensitivity was analysed by varying each parameter within the range of 10 % above and below its observed value (Table 7.5). The reference situation was a stable susceptible population and a selection pressure 1. The simulation outcome was measured by total seed bank size after 20 years of simulation. In most life history stages, the resistant biotype was more sensitive to a parameter change than the susceptible biotype. The variation in sensitivities is caused by the different effects of some model parameters on the different cohorts. The largest differences in sensitivity between the susceptible and resistant biotype were observed with the seed bank mortality between 10 and 20 cm deep. These two layers were, after ploughing, the main contributors to the seed reserve in the upper soil layer from which germination and emergence took place. The model was also quite sensitive to the germination probability, the seed production per plant and the fraction of produced seeds fallen from the plant and added to the soil seed bank. The model was less sensitive to seed bank mortality between 0-10 cm and between 20-25 cm, the natural mortality of germinated seeds, the natural mortality of plants, and the seed viability.

Table 7.5. Results of the sensitivity analysis of selected model parameters. Sensitivities indicate percent change in total seed bank size predicted per unit percent change in parameter value.

Parameter	Predicted sensitivities			
	Susceptible		Resistant	
	+	-	+	-
Mortality fraction seed bank				
0-5 cm	0.14	0.14	-	0.11
5-10 cm	1.68	2.02	1.49	3.56
10-15 cm	3.60	5.42	6.82	18.45
15-20 cm	3.90	6.04	7.29	21.82
20-25 cm	2.41	3.20	4.37	7.56
Germination probability	11.11	5.56	14.49	6.27
Natural mortality germinated seeds	4.73	8.21	4.20	6.76
Natural mortality plants	1.26	1.42	1.43	1.64
Final seed production	11.96	5.76	14.49	6.27
Fraction seed fall	11.96	5.76	14.49	6.27
Seed viability	5.25	5.76	6.19	6.26

Herbicide rotations

Besides the use of fitness differences between biotypes, another option to delay or manage herbicide resistant weed populations is the rotation of herbicides with a different mode of action. Jasieniuk *et al.* (1996) concluded that herbicide rotations were more effective in managing the herbicide resistant weeds when the resistance mutation was associated with a reduction in plant fitness in the absence of the herbicide. With our model, the frequency of triazine use which would not lead to an increase of the resistant population was estimated. The assumptions were that during the off-years the general kill rate as a result of a non-selecting weed control measure of the germinated susceptible and resistant seeds was 0.7 as before, and that the kill rate of susceptible and resistant plants consisted of a double application of a contact herbicide with a kill rate of 0.8 resulting in 0.96 effective kill of the first four cohorts. In the on-years, the kill rate of susceptible germinated seeds and plants of all cohorts was 0.96, while the kill rate of germinated resistant seeds was 0.7 and of resistant plants 0. In the year of triazine application, the selection pressure was 25. In Fig. 7.3 (upper graph), the development of the susceptible and resistant biotype is shown for triazine use once every 4 years. In this situation, weed control is performed according to a rotation with three off-years and one on-year. Within the 20 years of simulation, the size of the resistant population fluctuated strongly but increased in time while the susceptible population size slowly decreased. The importance of the fitness difference between triazine susceptible and resistant biotypes for managing the triazine resistant weed populations using herbicide rotations is also shown in Fig. 7.3. When the fitness of the triazine resistant biotype would have been equal to that of the susceptible

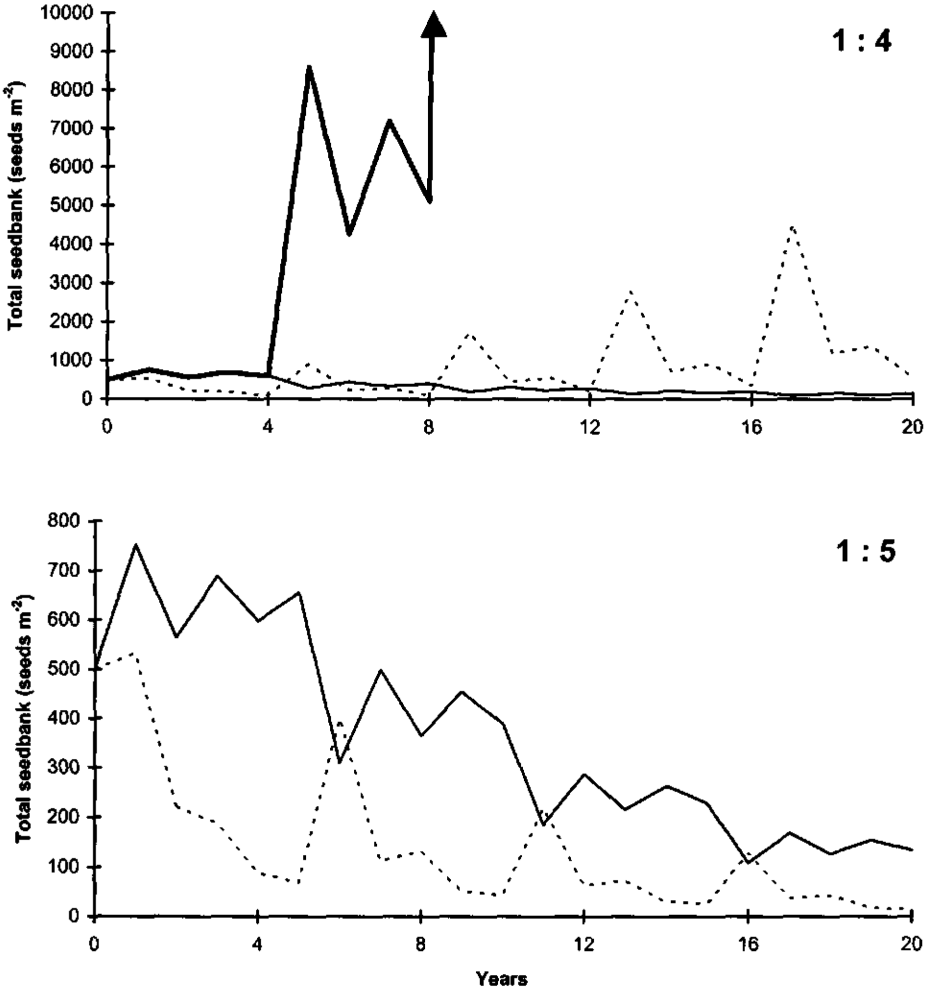

Fig. 7.3 Calculated population dynamics of triazine susceptible (——) and resistant (- - -) biotypes of *S. nigrum* in maize expressed as the total seed bank over 20 years depending on the frequency of triazine use. Thick line (upper graph) indicates the population dynamics of the resistant biotype when the fitness is similar to that of the susceptible biotype.

biotype, the herbicide rotation would hardly slow down the development of the triazine resistant weed population. Triazine applied once every 5 years (Fig. 7.3, lower graph) resulted in a slow decrease of the susceptible and resistant biotypes, with the resistant biotype disappearing slightly faster than the susceptible biotype. Although the absolute population size decreased, the resistance fraction in the population remained relatively constant over time (Fig. 7.4). In this case, population development depended on the balance between selection pressure and fitness differences between susceptible and resistant biotypes.

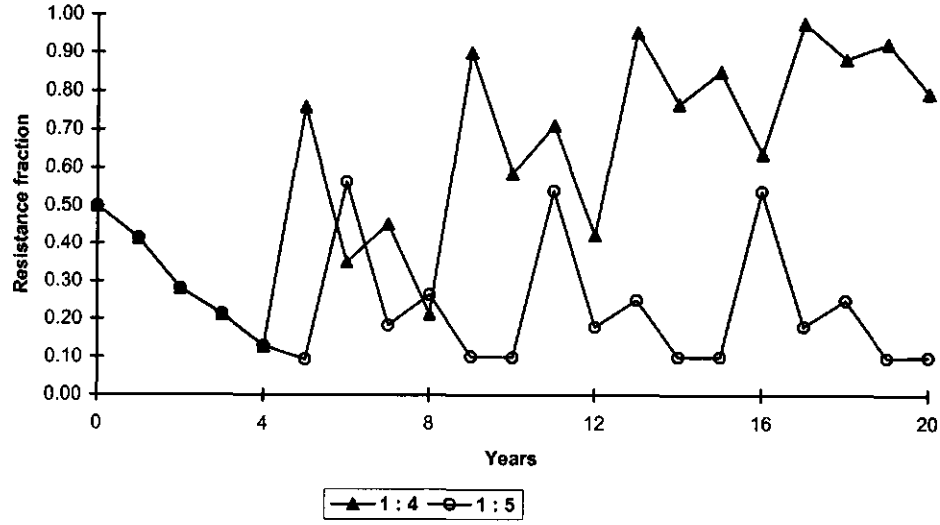

Fig. 7.4 The calculated pattern of the resistance fraction in the *S. nigrum* population in time when the triazine is used in different frequencies.

Conclusions and management options

The available knowledge integrated in this model showed the population dynamics from triazine susceptible and resistant biotypes of *S. nigrum* in the Dutch agro-ecosystem continuous cropping of maize. The model is very useful in the development of management scenarios, but is not validated with an independent data set. Experiments to validate the current findings should be the next step in the development of triazine resistance management strategies for practical purposes.

Simulation results showed that if no selecting herbicides were used, the emphasis of the weed control measures in maize should be put on controlling the susceptible biotype. This required, besides the control of plants, also sufficient control of germinated seeds which could be achieved by harrowing, hoeing or use of a soil applied herbicide with a different mode of action. In absence of the selecting herbicide, control of the susceptible biotype would result in the disappearance of the resistant biotype because of its reduced fitness. The main factors causing the reduced fitness of the resistant biotype appeared to be an increased seed mortality in the soil, a reduced seed production, and a lower number of seeds returning to the seed bank. compared to that of the susceptible biotype. The higher longevity of the susceptible seed bank may act as a buffer that delays the appearance of triazine resistance in the population (Gressel & Segel 1978). In the model, the earlier germination and subsequent eradication of the first cohort of resistant seeds in some years by a stale seedbed does not affect the long-term population dynamics. The advanced germination implies that the germinated resistant seeds from cohort 1 were killed by the seedbed preparation and did not contribute to the seed production. However, seeds from the other cohorts (2-5) emerged earlier compared to the emergence date of the crop and, therefore, had a competitive advantage. The decreased competition by the crop resulted in a higher total seed production compared to the regular emergence pattern of the resistant biotype, which easily compensated for the loss of the first cohort. However, the effect of a stale seedbed increases when the difference in the start of germination between biotypes increases.

An active strategy to eradicate the triazine resistant biotype from the population is the application of a herbicide with a selection pressure lower than 1, to which the resistant biotype is negative cross resistant, e.g. bentazone (Fig. 7.1; Van Oorschot & Van Leeuwen 1988, Claux *et al.* 1992). The resistant biotype is extra susceptible to these herbicides compared to the susceptible biotype and according to the model, eradication of the resistant biotype would take 6 years. This emphasizes the importance of seed bank dynamics in the development of resistance management strategies.

Despite the development of triazine resistance, in some cases it might be necessary or advisable to occasionally use triazines or other herbicides with the same mode of action to control other weeds. Herbicide rotations may then be useful tools to control persistent weed populations and to avoid the development or increase of resistance. In the optimal frequency of triazine use, the strong increase of the resistant biotype during the on-years has to be reduced by the lower relative fitness in the off-years. When no fitness difference between biotypes exists, herbicide rotations have a very small effect on the development of the resistant population. Simulation results showed that herbicides with the same mode of action might still be used if the frequency was once every 5 years or lower when no selecting herbicides are used in the off-years. However, waiting for a few extra years would have controlled the resistant biotype completely, which seems a much better option from a population dynamical point of view.

Applications of other crop management options in a system with continuous maize are difficult. Ghersa *et al.* (1994) suggested to sow the wheat crop earlier to control herbicide resistant *Lolium multiflorum*. In that case, competition with the crop was higher and selection for early germinating non-resistant weeds would take place. In Dutch maize cropping, the high minimum growth temperature of maize does not allow extra early sowing, and extra late sowing will result in a reduction in crop yield. Increase in weed suppression by the crop to increase the fitness cost in the resistant biotypes as suggested by Jordan (1993) for triazine resistant *Amaranthus hybridus*, might be another valuable management option. However, growth experiments at different low light levels showed no differences in Relative Growth Rate (RGR) between susceptible and resistant biotypes of *S. nigrum* (Kremer & Kropff 1998b, Chapter 4). This indicates that the biomass production of the susceptible and resistant biotype are reduced in competition with the maize crop, but that the effect of suppression by the crop is similar for both biotypes. An increase of the competitiveness of the maize crop seems, therefore, not to be an extra management tool in the control of triazine resistant *S. nigrum*.

Besides manipulation of the maize crop, other parts of the cropping system might also prove to be useful in the management of triazine resistant *S. nigrum*. Sowing of grass between the maize rows a few weeks after crop emergence to function as a catch crop of nutrients in autumn is practised in The Netherlands. This will increase the competition experienced by the weeds and also might increase the microbial activity in the soil, probably decreasing the seed persistence in the soil. Maize cropping in a no tillage system will also have these potential advantages but this will probably not compensate for the loss of soil cultivation practices in that system.

The suggested management strategies derived from our model, based on ecological data, support the suggestions by Gressel and Segel (1990). The best tactics to delay or manage triazine resistant weed populations are the reduction of the selection pressure of chemical weed control and the employment of mechanical cultivations in the cropping system as much as possible to exploit the ecological differences in the absence of selection.

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Chapter 8

General discussion

The evaluation of resistance management strategies based on fitness differences between biotypes in the absence of herbicides requires the determination of the relative fitness of triazine susceptible and resistant biotypes. Triazine resistance is the only proven case where the resistance trait comes with secondary effects, reducing the fitness in the absence of the herbicide. Knowledge of the components of relative fitness can be integrated by developing a model for the population dynamics of the weeds to determine the actual fitness of both biotypes under field conditions in the absence of the selecting herbicide. Such models can be useful tools to evaluate the effect of different management strategies on the development of triazine resistance in the *S. nigrum* population in continuous maize cropping.

Once the population dynamics of a relatively simple system with only one crop-weed-herbicide combination are better understood, conclusions on the most important life history processes determining fitness and on possible resistance management strategies might be generalized to other crop-weed-triazine combinations. Insight into the most important determinants of fitness might also be useful for research into potential fitness differences between weeds susceptible and resistant to other herbicide classes.

Population dynamics

The integration of different life history processes in a model in this study helped to quantify the reduction in fitness of the triazine resistant biotype of *S. nigrum* compared to the susceptible biotype in the agro-ecosystem of continuous maize cropping. The processes involved were germination, emergence, seed production, seed fall, and survival of seeds in the soil. Simulation results showed that the population of the susceptible biotype is difficult to maintain at stable levels in the long-term (98 % kill). The stabilization of the susceptible population with this high critical kill rate resulted in a decline of the resistant population, which disappeared after 11 years in the absence of the selecting herbicide. The lower fitness of the resistant biotype compared to that of the susceptible biotype in a maize crop is a result of the accumulation of differences between biotypes in the different individual life history processes. The differences between biotypes with respect to some important processes might be a point of application for the development of triazine resistance management strategies.

Germination. The simulation model shows the importance of germination for the population dynamics of *S. nigrum*. In the model, germination probabilities are assumed to be equal for both biotypes in spring at optimum soil temperatures but in early spring the dormancy of resistant seeds is lower than that of susceptible seeds. A difference in dormancy between triazine susceptible and resistant biotypes was also observed in other species in which however, results were contradictory (Mapplebeck *et al.* 1982, Weaver & Thomas 1986). The altered dormancy characteristics in early spring may also affect the secondary dormancy which is supposed to be induced by high soil temperatures (Roberts & Lockett 1978). The extent to which other maternal characteristics have an effect on the dormancy characteristic is still unclear. The reduced dormancy of the resistant seeds might also have consequences for the longevity of seeds in the soil.

The experiments showed a lower minimum germination temperature requirement of resistant seeds compared to susceptible seeds. In *Kochia scoparia*, the rapid germination of sulfonylurea resistant biotypes was based on a physiological mechanism (Dyer *et al.* 1993) and field observations indicated that an early spring cultivation combined with delayed crop sowing would specifically kill sylfonylurea resistant plants. In the case of triazine resistant *S. nigrum*, in cool springs when soil temperatures vary between the minimum germination temperature of both biotypes, early germinating resistant seeds will be killed during final seedbed preparations. The accelerated germination results, in the model, in a higher competitiveness of the resistant seedlings in the maize crop resulting in a higher seed production, compensating for the loss of early germinated seeds. However, if the period between onset of germination of resistant seeds and that of susceptible seeds increases, the effect of a stale seedbed increases. The opportunity to change the crop sowing date to further exploit the altered germination characteristics is very limited. The high temperature sum requirements needed to complete the maize growth cycle (Groot *et al.* 1986) does not permit a delay in the sowing date of maize, which is already late, only to control *S. nigrum*. Common soil temperatures in spring in The Netherlands do not result in a differentiation between the germination patterns of both biotypes.

Emergence. The number of germinated seeds, which successfully emerge from different depths, determines which fraction of the seed bank will produce plants and contribute to the reproductive success of the population. Resistant seeds emerged better from different depths than susceptible seeds, which was mainly determined by biotype and much less by a seed weight effect. This is the only investigated life history process where the resistant biotype performed better than the susceptible biotype. Since the difference in emergence characteristics between biotypes is not very large under controlled conditions, experiments under field conditions should be performed to prove the value of this characteristic.

Growth. The most measured and discussed aspect of fitness of triazine susceptible and resistant weeds is growth. The often reported reduced biomass production of the resistant biotype compared to that of the susceptible biotype was also found in our experiments. The growth analysis showed similar Relative Growth Rates (RGR) of both biotypes but a higher Specific Leaf Area (SLA) of the resistant biotype. The higher SLA might be a compensatory mechanism for the reduced photosynthetic efficiency of the resistant biotype. This is supported by the fact that chloroplasts of the resistant plants have an adaptive reorganization of the thylakoid components resembling that of shade type leaves (Lemoine *et al.* 1986). Curwiel and Van Rensen

(1998) suggested that the lower activity of photosystem II may indirectly have a signalling function on gene expression in the direction of shade-type chloroplasts. Curwiel *et al.* (1993) found a link between the shade type leaves and an increased sensitivity to photoinhibition of the resistant biotype of *C. album*. This indicates that triazine resistant biotypes may have mechanisms to compensate for the reduced photosynthetic efficiency such as increasing the SLA and possession of shade type leaves. A negative side effect is the increased sensitivity to photoinhibition at higher light levels which might explain the observed reduced biomass production of the resistant biotype compared to the susceptible biotype in the very young seedling phase. Relative growth rates were similar for both biotypes under controlled conditions and in the field. In the development of a triazine resistance management strategy this means that in general the triazine resistant biotype will have a reduced biomass production. The field experiment showed that interference of triazine susceptible and resistant biotypes of *S. nigrum* with the relatively open maize crop did not increase the fitness cost of the resistant biotype compared to a no cropping situation as was suggested by Jordan (1996).

Seed production. Seed production is an important aspect of fitness and is strongly related to biomass production. Although the fecundity index (seeds/biomass) was higher for the resistant biotype, the total seed production per plant was lower compared to that of the susceptible biotype of *S. nigrum* in a maize crop. The resistant biotype showed a relatively higher investment in seed production, probably compensating for a reduced biomass production, but this did not compensate for the higher biomass production of the susceptible biotype.

In a cropping system, for *S. nigrum* not only the total seed production is important but also the development rate. The sympodial flowering of *S. nigrum* causes a gradual ripening of the berries. In the model, only the ripe berries which fall on the soil surface end up in the soil seed bank and berries still attached to the plant are removed with the harvest. The lower development rate of the resistant biotype shows that, at the supposed time of harvest, a relatively lower fraction of the resistant berries have fallen on the soil surface. The combination of a lower fraction seed fall and a lower seed production of the resistant biotype compared to the susceptible biotype resulted in a 38 % reduction of the resistant seed input compared to susceptible seed input on a per plant basis.

In theory, management based on this fact should include harvesting the maize crop as early as possible so that only a small number of resistant berries have fallen from the plant. However, Mahn and Lemme (1989) showed the high plasticity of *S. nigrum* in growth and seed production and reported that ripe berries were already found 80 days after emergence. Therefore, complete prevention of seed input by an early harvest is impossible. In practice, the length of the maize growing season is already limited and cannot be reduced further. On top of that, harvested resistant *S. nigrum* seeds can survive ensiling and the digestive tract of animals resulting in possible spread of triazine resistant seeds to other fields. Occasionally, night frost can be observed before crop harvest. If the *S. nigrum* plants die off completely because of the frost, all the produced seeds will return to the seed bank and this will seriously affect the population dynamics but this scenario is not included in the model.

Seed longevity. The fate of seeds in the soil is the least investigated process in the population dynamics of triazine susceptible and resistant weeds. In the model, differences in seed mortality between both biotypes have a major effect on the population dynamics. Although data were based on a one year experiment, the survival of resistant seeds was substantially lower than that of susceptible seeds. This difference was much larger in the summer than in the winter. In the summer, germination and dormancy seemed to be the most important factors determining the seed mortality. The combination of reduced dormancy with a lower minimum germination temperature of resistant seeds compared to susceptible seeds may have been the cause of the large survival difference between biotypes. The differences in mortality can be very well exploited in a management strategy. However, more research should give insight into the long-term effects on seed survival of both biotypes and the possible linkage with the resistance trait.

Population dynamics model

The experiments and the simulation model can be very useful for the development of resistance management strategies. However, the results have been obtained with some assumptions and choices which should be discussed. The ability to draw legitimate conclusions on fitness differences between susceptible and resistant biotypes depends on the number of populations and the extent of the difference (Cousens *et al.* 1997). In most experiments, two populations were used to determine fitness differences. This to avoid problems with susceptible and resistant biotypes being adapted to different habitats and confounding of maternal phenotypic responses. The use of five different populations for the detailed growth analysis in Chapter 4 showed no effect of population on growth characteristics. Seed survival data were based on results from one population but the fact that in none of our experiments an interaction between population and biotype was observed and the quite large differences in seed survival gave an indication for the validity of the conclusions.

The simulation model integrates information on different life history processes but the experiments on these processes have been performed independently from each other. Ideally, the interaction between the processes should also be included in the model because variation in one process will affect the outcome of subsequent processes. However, for the comparison of fitness between susceptible and resistant biotypes, parameters were derived from data on realistic plants under proper conditions. Tests with independent data would be needed to evaluate the results of the model.

The model is based on population dynamic processes assumed to be important for the development of triazine resistance in a weed population. However, not all processes are included in the model. An important aspect of Dutch maize cropping is the application of manure where *S. nigrum* seeds can survive the digestive tract of animals (Elema & Scheepens 1992). This may cause potential problems with the spread of triazine resistant *S. nigrum* seeds between fields. Maxwell *et al.* (1990) did include seed input into their model but lack of experimental data kept us from introducing this into our model. The development of weed management strategies, e.g. exhaustion of the resistant seed bank, might be affected when resistant seeds are imported with manure.

The mortality and predation of seeds fallen on the soil surface is also not included in the model. Fallen seeds are assumed to be mixed through the top soil layer, but will remain on the soil surface for some time. Seed predation is usually small in agronomic systems due to intensive soil disturbance, seed burial by tillage, and lack of habitats for predators (Buhler *et al.* 1997). Seed decay by infections with fungi or other microorganisms is poorly understood yet (Kremer 1993). The lack of information on this process leads to the assumption that no differences between biotypes in fate of seeds exist in this part of the life history.

Data on the seed characteristics were measured on a time scale of one year. Long term dormancy cycles and survival rates of seeds were not included in the model. Such information would improve the validity of the simulation, but final conclusions of the simulations regarding management strategies are not to be expected.

The simulation results can be used for the development of triazine resistance management strategies and to determine on which part of the life cycle further research should focus. However, the model could not be validated with data. In the future, long-term experiments with fields containing triazine resistant *S. nigrum* on which different proposed management strategies are applied should be performed to evaluate the model. Besides triazine resistance development in the field, the model also shows the most important parts of the life cycle for the weed population dynamics. In the case of triazine susceptible and resistant *S. nigrum*, seed characteristics seemed to be the most important component in the life cycle. Further research into the longevity, dormancy, and germination of seeds from the susceptible and resistant biotype of *S. nigrum* seems to have the most perspectives.

Resistance management strategies

The reduced fitness of the resistant biotype compared to the susceptible biotype is very useful in the development of triazine resistance management strategies. Besides a reduction of the selection pressure, these strategies should be based on Integrated Weed Management. Powles and Matthews (1992) defined IWM as the use of a range of control techniques, embracing physical, chemical, and biological methods in an integrated fashion without excessive reliance on any one single method.

The prevention of herbicide resistance development is far more easier than the control of herbicide resistant weed populations. In a system with continuous cropping of maize, triazine resistance development can be prevented by a rotation of herbicides with different modes of action and an increase in the use of non-selecting weed control measures such as soil cultivation.

When triazine resistance is present, the most significant weed biological parameter determining fitness differences seems to be the seed characteristics of susceptible and resistant biotypes. The much lower longevity of resistant seeds compared to that of susceptible seeds can be the basis of a management strategy aimed at exhaustion of the resistant seed bank. The most important aspect of the depletion of the seed bank is the prevention of seed input. This can be achieved by a high kill rate of the weed control measures which should not include the use of triazines or herbicides with the same mode of action. This might be combined, when possible, with an advanced harvest date which will minimize seed fall and maximize removal of resistant seeds with the maize crop. To accelerate the mortality of resistant seeds in the soil, which have a lower dormancy than susceptible seeds, additional soil cultivations before crop sowing or after crop harvesting might be useful but the last option is not validated with experimental data at this time.

The incorporation of an increased competition by the crop into a management strategy seems to be less useful to specifically control the resistant biotype. Although the biomass and seed production will be reduced when competition by the crop is increased, this will not seriously select against the resistant biotype. More progress can probably be gained from specific chemical weed control measures.

A chemical weed control measure, not related to fitness differences between biotypes, is the application of a herbicide to which the resistant biotype is extra susceptible. An extra good control of the resistant biotype results in a selection pressure lower than 1 and an accelerated decline of the resistant population on top of the decline as a result of the lower fitness. The selection pressure can increase up to a value 3 before the increase of the resistant population compensates for the decrease of the resistant population because of its lower fitness compared to that of the susceptible biotype. In a herbicide rotation scheme, application of triazines should be limited to at least once every 5 years to control the resistant biotype.

Conclusion

The management strategies to control triazine resistant *S. nigrum* in the agro-ecosystem with continuous cropping of maize should include a combination of different factors. When chemical weed control is continued, selection pressure should be minimized by limiting the use of triazines or herbicides with the same mode of action, and the use of herbicides to which resistant biotypes are extra susceptible. The fitness difference between triazine susceptible and resistant biotypes of *S. nigrum* should be exploited by maximizing the use of weed control measures which do not select for a certain biotype, aiming at exhaustion of the resistant seed bank.

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Summary

The introduction of selective herbicides in agriculture about 50 years ago has led to a rapid adoption of chemical weed control technology by farmers in the industrial world. The success of herbicides is based on the high reliability and the low costs. However, the sole reliance on herbicides for weed control in cropping systems has resulted in a fast increase in the incidence of herbicide resistant weeds worldwide. In practice, triazine resistant weeds are effectively controlled by the use of alternative herbicides but the costs of weed control increases, the herbicide choice is limited because of cross resistance, and multiple resistance to herbicides with a different mode of action might develop. Therefore, non-chemical measures to control triazine resistant weeds are preferred.

The most promising ecologically-based strategy to control triazine resistant weeds is based on the utilization of the often observed fitness difference between triazine susceptible and resistant biotypes in the absence of the selecting herbicide. The components of fitness of a biotype are its survival and reproduction which both depend on environmental conditions and other biotypes in that environment. Often fitness effects are studied in relation to plant biomass or seed production. However, fitness is determined by the combination of different life history processes. Therefore, germination, emergence, growth, seed production, and seed longevity of triazine susceptible and resistant biotypes of black nightshade in an agro-ecosystem with continuous cropping of maize were studied and integrated in a population dynamics model. This model can be a useful tool to evaluate the effect of different management strategies on the development of triazine resistance in the *S. nigrum* population in continuous maize cropping.

In Chapter 2, the seedling emergence patterns in time of triazine susceptible and resistant *Solanum nigrum* in the field were studied. Emergence patterns of the populations were similar in one year, but in another year resistant seedlings emerged faster and the number of resistant seedlings was higher. To explain emergence patterns, a germination experiment was carried out. Experimental results showed a lower dormancy of the resistant seeds compared to that of susceptible seeds in early spring. Minimum germination temperature requirements were also lower for resistant seeds than for susceptible seeds enabling resistant seeds to germinate earlier in the season depending on soil temperatures.

The emergence of germinated susceptible and resistant seeds from different soil depths is described in Chapter 3. The emergence fraction increased with temperature and decreased with increasing depth of placement. Resistant seeds showed a higher emergence fraction than susceptible seeds while emergence rate of resistant seeds was slightly lower. The emergence fraction appeared to be highest at intermediate seed weights.

The effect of light intensity on growth of plants from five different populations of different origin of triazine susceptible and resistant *Solanum nigrum* was studied in

growth chambers at three light levels (Chapter 4). Plants were grown without competition and with optimal mineral nutrition. After 29 days, biomass of the resistant biotypes was about 25 % less than that of the susceptible biotypes at each light level. Classical growth analysis showed that this was the result of a lower initial biomass of the resistant biotype at the start of the experiment as the relative growth rates (RGR) of the susceptible and the resistant biotype in the early growth phase were equal. The most important growth component accounting for the RGR was the Specific Leaf Area, which was higher for the resistant biotype but which was compensated by a lower Net Assimilation Rate (NAR). The fraction dry matter invested in leaves was equal for both biotypes but the resistant biotype produced more leaf area per unit leaf weight.

In Chapter 5, biomass and berry production of triazine susceptible and resistant *Solanum nigrum* in the field with and without a maize crop were studied during three years. Delayed transplanting reduced the final dry matter production and berry production of both biotypes strongly. In most of the experiments, the final total dry matter production and berry production of the triazine resistant biotype were lower than those of the susceptible biotype. However, relative growth rates of both biotypes in a maize crop seemed to be similar during the exponential growth phase. An important determinant of final dry matter and berry production could be dry matter accumulation in the very young seedling phase. The presence of a maize crop did not affect the fitness costs in triazine resistant *S. nigrum*.

The fate of seeds from triazine susceptible and resistant *S. nigrum* in the soil was studied during the growing season and outside the growing season, and described in Chapter 6. The highest seed mortality occurred during the growing season after which only 28 % of the resistant seeds and 48 % of the susceptible seeds remained viable. The number of emerged seedlings was similar for both biotypes. The highest difference in seed mortality between biotypes occurred at depths between 6 and 15 cm. The difference between the two biotypes could probably be partly attributed to a higher fatal germination of the resistant seeds because of the lower minimum germination temperature requirement compared to that of the susceptible seeds. However, more factors must have been involved. Seed mortality outside the growing season was lower than 4 % for both biotypes.

A population dynamics model was developed to quantify fitness differences between triazine susceptible and resistant *S. nigrum* in an agro-ecosystem with continuous cropping of maize (Chapter 7). The model was parameterized with experimental data on different life history processes such as germination, emergence, growth, seed production, and seed persistence in the soil reported in this thesis. In continuous cropping of maize, fitness of the resistant biotype was much lower than of the susceptible biotype, mainly caused by differences in seed characteristics between biotypes. The effect of different weed control measures and their selection pressure on the population dynamics of triazine susceptible and resistant *S. nigrum* in maize was studied. Triazine resistance management strategies based on fitness differences between susceptible and resistant biotypes have to focus on exhaustion of the seed bank of the resistant population.

In the general discussion (Chapter 8), the differences between biotypes in the different life history processes and their potential for the development of resistance management strategies are discussed. Results from the population dynamics model, which integrated the different life history processes, are evaluated and management strategies are proposed. Scenarios to control triazine resistant *S. nigrum* in a continuous cropping of maize should include a reduction of the selection pressure of chemical weed control measures, e.g. rotations of herbicide or decreasing the herbicide dose. The observed fitness difference between biotypes should be exploited by maximizing non-selective weed control measures such as soil cultivation, preferably aimed at depletion of the seed bank of the resistant biotype.

Samenvatting

De introductie van selectieve herbiciden in de landbouw ongeveer 50 jaar geleden heeft geresulteerd in een snelle acceptatie van de chemische onkruidbestrijding door boeren in de geïndustrialiseerde wereld. Het succes van herbiciden is gebaseerd op de hoge betrouwbaarheid en de lage kosten. Het volledig vertrouwen op herbiciden voor onkruidbestrijding in teeltsystemen heeft geresulteerd in een snelle toename van het aantal resistente onkruiden wereldwijd. Herbicide resistente onkruiden worden in de praktijk effectief bestreden met het gebruik van alternatieve herbiciden maar de kosten van onkruidbestrijding stijgen, de herbicidekeuze is gelimiteerd vanwege kruisresistentie en er kan zich multipele resistentie tegen meerdere herbiciden met verschillende werkingsmechanismen ontwikkelen. Om deze redenen is een niet-chemische bestrijding van resistente onkruiden gewenst.

De meest veelbelovende ecologische gefundeerde manier om triazine resistente onkruiden te bestrijden is om gebruik te maken van het vaak waargenomen fitness verschil tussen triazine gevoelige en resistente biotypes in de afwezigheid van het selecterende herbicide. Fitness van een biotype bestaat uit overleving en reproductie die beide afhangen van de groeicondities en andere biotypes in de omgeving. Vaak zijn fitness effecten bestudeerd in relatie tot de biomassa- of zaadproductie van een biotype. Fitness is echter bepaald door de combinatie van verschillende processen in de levenscyclus. Om deze reden zijn kieming, opkomst, groei, zaadproductie en zaadoverleving van triazine gevoelige en resistente biotypes van zwarte nachtschade in een agro-ecosysteem met continuteelt maïs bestudeerd en geïntegreerd in een populatie dynamisch model. Dit model kan een waardevolle bijdrage leveren om het effect van verschillende managementstrategieën op de ontwikkeling van triazine resistentie in de *S. nigrum* populatie in een continuteelt maïs te evalueren.

In hoofdstuk 2 is het opkomstpatroon van triazine gevoelige en resistente *S. nigrum* in het veld bestudeerd. Opkomstpatronen van beide populaties waren gelijk in het ene jaar, maar in het andere jaar kwamen de resistente kiemplanten sneller op en in een groter aantal dan de gevoelige kiemplanten. Om de opkomstpatronen te verklaren is een kiemingsexperiment uitgevoerd. Proefresultaten toonden een lagere kiemrust van resistente zaden vergeleken met dat van gevoelige zaden in het vroege voorjaar. De minimum kiemtemperatuur van resistente zaden was ook lager dan die van gevoelige zaden wat kan resulteren in een vroegere kieming van resistente zaden wat afhankelijk is van de bodemtemperatuur.

De opkomst van gekiemde gevoelige en resistente zaden van verschillende bodemdieptes is beschreven in hoofdstuk 3. De opkomstfractie nam toe met toenemende temperatuur en afnemende begraafdiepte. Resistente zaden vertoonden een betere opkomst dan gevoelige zaden, maar hun opkomstsnelheid was iets lager. De opkomstfractie was het hoogst bij de gemiddelde zaadgewichten.

Het effect van lichtintensiteit op de groei van planten van vijf verschillende herkomsten van triazine gevoelige en resistente *S. nigrum* is bestudeerd in fytotrons onder drie lichtniveaus (hoofdstuk 4). Planten zijn opgegroeid zonder competitie met een optimale nutriëntenvoorziening. Na 29 dagen was de biomassa van resistente planten ongeveer 25 % lager dan die van gevoelige planten onder elk lichtniveau. Een klassieke groeianalyse toonde aan dat dit het resultaat was van een lagere initiele biomassa van het resistente biotype bij de start van het experiment omdat de relatieve groeisnelheid van het gevoelige en het resistente biotype gelijk waren in de fase van vroege groei. De belangrijkste groeiparameter voor de relatieve groeisnelheid was de Specific Leaf Area (SLA) welke hoger was voor het resistente biotype maar wat gecompenseerd werd door een lagere Net Assimilation Rate (NAR). De fractie droge stof die geïnvesteerd is in bladeren is gelijk voor beide biotypes maar het resistente biotype produceerde meer bladoppervlak per eenheid bladgewicht.

In hoofdstuk 5, is de biomassa- en zaadproductie van triazine gevoelige en resistente *S. nigrum* onder veldomstandigheden met en zonder een maïsgewas bestudeerd gedurende drie jaren. Het later verplanten van beide biotypes in een maïsgewas verlaagde de uiteindelijke biomassa- en zaadproductie sterk. In de meeste experimenten was de uiteindelijke biomassa- en zaadproductie van het resistente biotype lager dan dat van het gevoelige biotype. De relatieve groeisnelheden van beide biotypes in een maïsgewas schenen echter gelijk te zijn in de exponentiële groeifase. Een belangrijke factor voor de uiteindelijke biomassa- en zaadproductie zou de droge stofproductie in de zeer jonge kiemplantfase kunnen zijn. De aanwezigheid van een maïsgewas veranderde de fitness kosten van het resistente biotype niet.

Het lot van zaden van triazine gevoelige en resistente *S. nigrum* in de bodem is bestudeerd tijdens en buiten het groeiseizoen en beschreven in hoofdstuk 6. De hoogste zaadmortaliteit trad op gedurende het groeiseizoen waar na afloop slechts 28 % van de resistente zaden en 48 % van de gevoelige zaden levensvatbaar gebleven waren. Het grootste verschil in zaadmortaliteit tussen biotypes werd gevonden op een diepte tussen 6 en 15 cm. De verschillen tussen de biotypes kan mogelijk worden toegerekend aan een hogere fatale kieming als gevolg van een lagere minimum kiemtemperatuur van de resistente zaden vergeleken met die van de gevoelige zaden. Er zijn echter meerdere factoren die invloed hebben. Zaadmortaliteit buiten het groeiseizoen was lager dan 4 % voor beide biotypes.

Een populatie dynamisch model is ontwikkeld om de fitness verschillen tussen triazine gevoelige en resistente *S. nigrum* in een een agro-ecosysteem met continu maïs te kunnen kwantificeren (hoofdstuk 7). Het model is geparameteriseerd met proefgegevens van de verschillende levenscyclusprocessen zoals kieming, opkomst, groei, zaadproductie en zaadoverleving in de bodem zoals beschreven in dit proefschrift. In een continuteelt van maïs, de fitness van het resistente biotype is veel lager dan dat van het gevoelige biotype wat vooral wordt veroorzaakt door verschillen in zaadkarakteristieken tussen beide biotypes. Het effect van verschillende onkruidbestrijdingsmaatregelen en hun selectiedruk op de populatie dynamica van triazine gevoelige en resistente *S. nigrum* in maïs is bestudeerd met het model. Triazine resistentie management strategieën gebaseerd op de fitness verschillen tussen gevoelige en resistente biotypes moeten worden gericht op een uitputting van de zaadbank van de resistente populatie.

In de algemene discussie (hoofdstuk 8), worden de verschillen tussen biotypes in de verschillende levenscyclusprocessen en hun mogelijkheden voor de ontwikkeling van resistentie management strategieën besproken. Resultaten van het populatie dynamisch model, welke de verschillende processen integreert, worden geëvalueerd and management strategieën worden besproken. Scenarios om triazine resistente *S. nigrum* in een continuteelt maïs te bestrijden zouden moeten bestaan uit een reductie van de selectiedruk zoals het gebruik van een herbiciderotatie of een reductie van de herbicide dosis. Het waargenomen fitnessverschil tussen biotypes zou kunnen worden toegepast door een maximaal gebruik van onkruidbestrijdingsmaatregelen die niet selecteren voor het resistente biotype. De meest interessante optie lijkt een intensivering van grondbewerking te zijn die is gericht op uitputting van de zaadbank van de resistente populatie.

Curriculum vitae

Eddie Kremer werd geboren op 16 juni 1968 te Hardenberg. In mei 1986 behaalde hij het VWO diploma aan de Jan van Arkel te Hardenberg. Aansluitend werd aangevangen met de studie Landbouwplantenteelt aan de Landbouwuniversiteit in Wageningen. Tijdens zijn doctoraalstudie doorliep hij afstudeervakken op het gebied van de akkerbouw en dat van de onkruidkunde waarna hij in juni 1992 afstudeerde. In maart 1992 begon hij als assistent in opleiding aan de toenmalige vakgroep Vegetatiekunde, Plantenoecologie en Onkruidkunde van de Landbouwuniversiteit in Wageningen. Na enkele naamswijzigingen werd de leerstoel Onkruidkunde verplaatst naar de vakgroep Theoretische Productie Ecologie waar hij zijn promotieonderzoek voortzette. Zijn onderzoek betrof de populatiedynamica van triazine gevoelige en resistente biotypes van het onkruid zwarte nachtschade in maïs. De resultaten van dit onderzoek onder leiding van de promotoren prof. dr. M.J. Kropff, prof. dr. ir. P.C. Struik en co-promotor dr. L.A.P. Lotz zijn vastgelegd in dit proefschrift. Sinds september 1998 is hij werkzaam als zaadtechnoloog/productontwikkelaar bij Novartis Seeds in Enkhuizen.