VECTOR CONTROL OF SCHISTOSOMIASIS USING NATIVE AFRICAN PLANTS

SEMINAR

Brussels, 24 March 1992

PROCEEDINGS EDITED BY

J. J. Symoens, S. Geerts & L. Triest

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ROYAL ACADEMY OF OVERSEAS SCIENCES

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FOREWORD

Schistosomiasis (or bilharzia) is a very widespread disease in the tropics. The estimated number of cases is 200 million and 500-600 million people are considered at risk in 76 developing countries. The infection has a relatively low mortality rate but a high morbidity rate and causes severe debilitating illness in millions of people.

As the disease is transmitted from infected individuals to susceptible persons via an intermediate snail host, it seems of importance to examine to what extent vector control can reduce the prevalence of the disease and by which measures, easily applied in developing countries, such control could be completed.

Therefore, on the proposal of Prof. Dr. J.J. Symoens, the Section of Natural and Medical Sciences of the Royal Academy of Overseas Sciences organized a Seminar Meeting on the theme of «Vector control of schistosomiasis using native African plants».

This meeting was held in Brussels on 24 March 1992 and brought together about 40 participants.

The Academy wishes to express its sincere thanks to Prof. Dr. S. Geerts (Institute of Tropical Medicine, Antwerp) and Dr. L. Triest (Laboratory of General Botany and Nature Management, Free University of Brussels) for their contribution to the organization of the Seminar and the editing of the present Proceedings, as well as to Prof. Dr. J. Mortelmans (Institute of Tropical Medicine, Antwerp) and Prof. Dr. J. Duncan (Hitchin, England) who chaired the sessions of the meeting.

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THE CONTROL OF SNAILS AND SCHISTOSOMIASIS

BY

A. M. POLDERMAN *

SUMMARY. — In this introduction to the seminar some aspects of the population biology of schistosomes are discussed. Both on theoretical grounds and based on experience in the field, it can be concluded that reduction of transmission through snail control will often fail to result in reduced levels of infection and morbidity. Moreover, snail control requires a financial input and a level of organization which are often absent. Snail control is therefore unlikely to play a central role in the control of schistosomiasis, in most places. Yet, in intensive efforts of integrated control, efforts to reduce snail populations may complement other control measures. From a public health education point of view, too, snail control may not be neglected. Molluscicides to be used in that context have to meet other requirements.

RÉSUMÉ. — *La lutte contre les mollusques et la schistosomiase.* — Dans la présente introduction à la journée d'étude, quelques aspects de la biologie des populations de schistosomes sont discutés. Aussi bien sur la base de considérations théoriques que de l'expérience sur le terrain, on peut conclure que la réduction de la transmission par une lutte contre les gastéropodes vecteurs ne conduira, bien souvent, pas à une réduction du taux d'infection ni de la morbidité. En outre, la lutte contre les gastéropodes nécessite un effort financier et un niveau d'organisation qui font souvent défaut. En conséquence, dans la plupart des endroits, la lutte contre les gastéropodes ne semble pas devoir jouer un rôle important dans la mosaïque des méthodes de lutte. Toutefois, dans les efforts intensifs de lutte intégrée, les tentatives visant à réduire les populations de gastéropodes peuvent être complémentaires aux autres mesures de lutte. Du point de vue sanitaire également, la lutte contre les mollusques ne doit pas être négligée. Dans ce contexte, les molluscicides à mettre en œuvre devront satisfaire à de nouvelles exigences.

SAMENVATTING. — *Slakkenbestrijding en schistosomiasis.* — In deze inleiding tot de studiedag worden enkele aspecten van de populatiebiologie van schistosomen besproken. Uit zowel theoretische overwegingen als praktijkervaring volgt dat het onwaarschijnlijk is dat vermindering van transmissie door slakkenbestrijding in het

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algemeen leiden zal tot vermindering van infectie-niveau en van de morbiditeit. Bovendien vergt slakkenbestrijding een aanzienlijke financiële en organisatorische inzet die veelal niet beschikbaar is. Slakkenbestrijding zal daarom in het algemeen een bescheiden plaats innemen in het complex van bestrijdingsmaatregelen. Als onderdeel van een geïntegreerde bestrijding, en ook in verband met gezondheidsvoorlichting voor de infectiepreventie, zullen slakkenbestrijdingsmaatregelen onmisbaar zijn. Een dergelijk gebruik van molluscic iden stelt andere eisen aan—nieuw te ontwikkelen—molluscic iden.

* * *

In the opening session of this workshop on the role of using native African plants to control the schistosomes' intermediate host, some basic concepts of the epidemiology and of the vulnerability of various parts of the life cycle should be briefly discussed. Before embarking on efforts to control schistosomiasis, we could learn a lot from the parasite's mechanisms to maintain stable populations in man. A number of—sometimes rather personal—observations and considerations are given in this introduction.

Snail control and disease control

Control of schistosomiasis aims, first of all, at controlling a disease; in this sense the ultimate goal is morbidity control. Morbidity is thought to be related to the population's infection load. Infection control will therefore be a prerequisite for morbidity control. Reduction of the level of infection of man, recognized by declining prevalences and egg-excretion, can be achieved by reducing transmission. Transmission control is, without any doubt, the basis of morbidity control. The relation between transmission and morbidity, however, is complex, and reduced transmission will not necessarily result in reduced morbidity. This will be illustrated below. Consequently, the impact of particular ways of transmission control should not only be evaluated on the basis of the intrinsic efficacy of the measures taken, but also in terms of other parameters, more closely linked to the health of the human population. The most direct way to test the activity of a molluscicide is to check whether it kills snails, but to test its contribution in schistosomiasis control, its role in reducing disease in the infected population should be evaluated.

Morbidity is rather variable in schistosomiasis, and changes in morbidity brought about by control measures, are difficult to quantify. Instead, the prevalence and intensity of infection in man are often used to quantify the amount of infection in man. Although at the population level, and in stable endemic situations, a certain correlation has repeatedly been demonstrated between the amount of infection and morbidity, little and conflicting evidence exists on the relation between both variables after disruption of the balance between parasite and host through control measures. Assessment of the impact of control measures (such as snail control) on morbidity remains unsatisfactory and that is why other, indirect parameters continue to be used.

Epidemiological considerations

In endemic situations a characteristic relation between prevalence, intensity of infection and age is found, with peak-prevalences and intensities of infection at ages of 10-15 years. This is true in areas with a very high 'overall prevalence' and also in areas with much lower prevalences. In a stable endemic locality, that is a focus in which the intermediate host is transmitting the infection more or less continuously, everybody is likely to get infected. Remaining «uninfected» has to be explained in one of the following ways. 1) Some may escape infection because of a different way of life; 2) many infections remain undiagnosed as a consequence of the relative unsensitivity of the diagnostic procedures; 3) many seemingly homogeneous endemic areas consist, in fact, of a patchwork of foci with and without transmission; and 4) some inhabitants of the endemic focus may be or become immune to (renewed) infection, although it remains doubtful whether full immunity is ever reached. Finally, transmission that changes with time and immigration of non-infected subjects from non-endemic areas play a role as well.

The lack of sensitivity of the current diagnostic procedures is indeed quite important. Serological and anecdotal evidence, supported by the use of statistical approaches to estimate the fractions of microscopically false negative subjects, suggest that the real prevalence may well be two or even three times as high as the observed prevalence (DE VLAS *et al.* 1992).

Schistosomiasis has the reputation of being a focal disease. Transmission is limited to certain foci, sometimes because the intermediate hosts cannot survive elsewhere, sometimes because man's exposure is concentrated in specific sites. In some extreme situations the underlying reasons for focality are well understood (e.g. POLDERMAN *et al.* 1985) but mostly the patchy pattern of transmission is difficult to recognize and even more difficult to explain (VERA *et al.* 1992).

The presence or absence of intermediate hosts is an unsatisfactory tool to recognize focality of transmission. Rather than searching snails, and then determining whether these are infected, transmission sites may be recognized by first identifying foci with high prevalences in man and then trying to search for habitats harbouring (infected) snails. Often, however, no snails, or no infected snails are to be found and we can only guess the site of transmission. For example, a clear-cut correlation with sex among children in Gorgora, along the shores of lake Tana in Ethiopia, was suggestive for a different pattern of transmission as compared to the neighbouring inland villages, indicating that transmission takes place in the lake itself. However, only very few specimens of *B. pfeifferi* and no infected snails were found during monthly surveys (POLDERMAN 1975). In parts of the Benue Valley, northern Cameroon, *S. haematobium* prevalences of 10-40% are found but in spite of extensive and longitudinal field research for over two years, the intermediate host could so far not been identified with certainty! (SLOOTWEG, pers. comm.). On the other hand, the massive quantities of *B. pfeifferi* and the much smaller numbers of *Bulinus spec.* present in waterbodies in the Dogon area, Mali, are in marked contrast with the high prevalence of *S. haematobium* and the scarcity of *S. mansoni* in the area (GRYSEELS, pers. comm). In short, the presence of (infected) snails is a very unsatisfactory tool for the identification of transmission foci.

Another conclusion to be drawn from the above examples is that very few snails, and snails that are present for very limited periods of time only, can be responsible for considerable prevalences. In the Ethiopian village of Gorgora (prevalence in children: > 85 %), snails are likely to be present for sufficient time to transmit the infection, in some years only. In the Sahel zone of northern Cameroon too, the transmission would seem to be limited to short periods in small temporary water bodies (GREER *et al.* 1990). Many of these temporary water bodies contain water for several weeks or a few months a year only. More direct evidence is to be obtained from the exposure of travelers to endemic areas. Recently two groups of Dutch travelers visited Mali's Dogon area. Each group rested for an hour or so at a small pool considered free of schistosome transmission because of its location just downstream a waterfall. Of the 28 persons who swam, 27 became infected! Ten of them harboured more than one species of schistosomes and 5 excreted more than 100 eggs per gram (VISSER, STUIVER & POLDERMAN 1992). The prevalence of schistosomiasis among the resident population around this pool is not known but it cannot be much higher than the 96% recorded among the travelers who visited the area for one day only!

In most studies on the relation between prevalence of infection and exposure to infested water, an association of high prevalences and intense water contacts is seen in children and adolescents. This association, however, does not imply a causative relation. When trying to understand the pattern of *S. mansoni* infection in northern Ethiopia, I was first impressed by observing similar age-specific curves in water contacts and in prevalence of infection. Only thereafter it transpired that this was true for male muslims, who had very little exposure and for female christians who were much more exposed. Yet the levels of infection in both groups was similar (POLDERMAN, 1975). A clear-cut relation between exposure and prevalence or intensity of infection has only been described in a few studies and in specific age groups (WILKINS *et al.* 1987).

The regulation of the parasite populations

All these observations indicate that limited exposure can result in high prevalences and wormloads. Additional exposure will not lead to ever-growing worm populations. The implication is that, in areas with more than marginal transmission, parasite populations are largely regulated by the host's immune response rather than by transmission factors. This concept has been elegantly worked out by BRADLEY (1972), although the mechanisms of regulation by the host's immune response were largely unknown at that time. In recent years, results of experimental work as well as field observations became available to support the concept of a regulatory role of immunity in schistosomiasis (e.g. HAGAN *et al.* 1987). Although many aspects of the dynamics of infection and reinfection after treatment remain unexplained, an important feature of this immunity is that it is built up very gradually, in the course of years. It explains why very high intensities of infection can be built up in children (over 30,000 eggs in some children in Maniema, Zaire and in Richard Toll, Senegal) (POLDERMAN 1984; F. STELMA *et al.* 1992) and why reinfection after treatment is generally very rapid in children but not in adults. The consequences for our interference with the parasite's life cycle are considerable. This can be illustrated with the following theoretical example.

The enormous reproductive potential of schistosome has been nicely illustrated by STURROCK (1974).

Table 1

The reproductive potential of St. Lucian Schistosoma mansoni

IN MAN	
Eggs/adult female/day	100
Life-span of female worm in days	1,000
Total numbers of eggs produced:	100,000
IN SNAIL	
Cercariae/snail/day	1,373
Life-span of infected snails in days	182
Total number of cercariae produced	250,000
Potential number of F_1 adults:	**23,000,000,000**

A most pertinent conclusion, however, was not drawn: in a stable endemic situation only two worms of the total offspring of one couple will survive; the other potential 25 billion minus two, will not succeed to develop into egg producing adults. There must indeed be other forces than the transmission-dependent parameters to achieve this type of equilibrium. In the other way round, reduction of the faecal pollution or of the density of the snail population with e.g. 50% is not likely to result in a proportional reduction of the parasite load in man, when those other stabilizing forces are in operation.

The expectations of the impact of interventions at the level of transmission ought to be considered in the light of the considerations sketched above. In many endemic situations a surplus of transmission is likely to exist. There, reduced faecal pollution, snail densities or water contact will not automatically result in reduced infection in man. Only beyond a certain threshold, reduction of transmission will result in reduced infection in man. The level of this threshold is not known but several examples suggest that near to perfect intervention has to be accomplished to result in reduced transmission. Population based chemotherapy may produce a dramatic decrease in a population's egg output but reduced infection rates in snails or reduced reinfection of man have only rarely been demonstrated (GRYSEELS & POLDERMAN 1992). Similarly, snail control greatly reduces the number of snails in a population but evidence on reduction of the incidence of human infection is scarce and often unconvincing.

The threshold is unlikely to be reached by employing one single type of intervention: generally, snail control will only be effective in controlling infection in man, if it is combined with other control measures. The contribution of the various components of an integrated approach to control is therefore difficult to measure. Indeed, the reported impact of snail control on the infection in man could often be attributed as well to other measures that were implemented simultaneously (GILLES *et al.* 1973).

Snail control

Snail control can be achieved in a number of different ways: modulation of the snail's habitat, biological control using for instance competing or predating species of snails or fish, and the use of specific molluscicidal products. Many chemical and plant-derived compounds have been used for their molluscicidal properties. These compounds differ in molluscicidal potential, in toxicity to non-target organisms, in stability in various types of water body, in applicability and bio-degradability. To safeguard the environment from unforeseen side-effects, to minimize logistic problems in molluscicide application, and of course to improve molluscicidal potency without increasing toxicity, much research has been carried out and more research is certainly needed.

Some molluscicides are definitely more potent than others. The results obtained with the best molluscicides available have not prevented the general philosophy of schistosomiasis control to shift from snail control to morbidity control based on treatment of the infected subjects. Why has this happened? Were the best available molluscicides not good enough? Was the concept of preventing infection through snail control not sound? Or rather, are other types of control easier to perform or more cost-effective? And then: what is to be expected from new, alternative molluscicides? How could they be used in a sustainable system of health care and prevention of disease? These questions are at the basis of today's workshop.

Although we will not be able to give an answer to all of them, some remarks have to be made on the different ways in which molluscicides are used and on the advantages and problems in the application of molluscicides in control programmes.

Area-wide and focal snail control

Two conceptually different types of snail control can be distinguished: area-wide control and focal snail control.

The concept of area-wide snail control is clear. It is attempted to eradicate the vector snails from an area that is well demarcated. Accepting that real eradication will normally be impossible, it will at least be attempted to reduce the snail populations to levels that are so low that repopulation takes place slowly, and that repetition of the molluscicide application can be infrequent, e.g. once every 4-6 months. Ideally, the low frequency of repeated application should render the approach financially affordable. The requirements for area-wide snail control to be successful include the treatment of a sufficiently large area to minimize the effect of inmigrating snails, an organizational capacity to treat all water bodies and the availability of funds to properly carry out treatments and monitoring. The requirements clearly indicate the limitations of this approach. Generally considerable masses of water have to be treated and the costs of both the organization and the application of molluscicides as well as of the molluscicides themselves, are high. In Fayoum, Egypt, for instance, with a human population of over 1.6 million, 40,000 km of canals and drains, have to be treated regularly. During the years 1969-1971, when control was mainly based on area-wide molluscicid-ing in this area, this required 90-130 metric tons of Bayluscide a year and teams totalling some 400 snail collectors and applicators of molluscicides were involved (ABDEL-WAHAB 1982).

Focal molluscicidіng is, essentially, based on the impressively focal nature of transmission. When transmission is not taking place everywhere, why should large amounts of molluscicides be wasted by homogeneously treating large areas? Although this concept would seem to be essentially correct, it appears very difficult to determine where transmission takes place and where it does not. It is an interesting experience to visit an endemic area with a group of 'experts in schistosomiasis'. While searching for the principal foci of transmission and discussing the length of the stretches of the irrigation canal or river to be treated, overwhelming disagreement will be observed: the experienced eye is not to be trusted in localizing the transmission foci and in identifying the extent to which focal mollusciciding is required. Moreover, the foci are likely to change from one year to the other. A lot of observations and experiments in each particular endemic area are required before conclusions on transmission drawn from superficial examination of a particular spot, can be relied on (e.g. ADEWUNMI *et al.* 1991).

The selection of foci to be treated can be based on: 1) the presence of snails, 2) the presence of water contacts or 3) the presence of infection in man. Normally combinations of 1) and 2) are used to select the sites to be treated. Although the approach would seem logical, there are hardly any convincingly successful examples. Comparison of the rate of reinfection after praziquantel treatment in villages with and without focal mollusciciding, gave little or no difference in experiments in Zaire (POLDERMAN 1984), Burundi (GRYSEELS 1990) and Mali (WERLER 1989). The objection that the wrong or insufficient habitats may have been treated is not to the point. Or rather, it stresses the very nature of the problem: even in those types of research settings it appears impossible to adequately estimate the intensity, frequency and spots at which focal mollusciciding should take place to achieve results in terms of reduced rates of reinfection after chemotherapeutic intervention. There are hardly any examples of parasitologically proven success in focal mollusciciding (KLUMPP & CHU 1987).

Snail control in years to come

Even though the examples given indicate that attempts to achieve control of schistosomiasis through snail control have often been unrewarding, and even though the lack of success to achieve lasting results can in fact be predicted on population-biological grounds, it can not be denied that snail control has a contribution of its own in controlling the disease.

First of all, lasting control can probably only be achieved through an integrated approach, combining different methods of control. Environmental changes, radical improvement of water provision and excreta disposal, increased public awareness, and adequate facilities to diagnose and when necessary to treat infected patients will be the cornerstones of control. Snail control will be another element in such an integrated effort. In a situation with limited transmission, even a marginal reduction of snail populations may contribute to reduce transmission.

Secondly, even in less evolved situations, molluscicides play a very important role in public health education. Once the snail's role in the life-cycle has been recognized, it is all too logical that they have to be destructed. Attempts to change man's way of life and at the same time to allow the snails to continue to infest the water bodies, are incompatible. In this context the first requirements of a molluscicide are not its molluscicidal potency but rather the possibilities to include its use in the daily life. Locally produced molluscicides that are applied focally in the main contact points would meet these requirements.

Although the use of molluscicides has resulted in a great number of failures and wastes of energy and funds, molluscicides are likely to remain an important tool in control. Further research on alternative molluscicides that suit the particular requirements for use in maintenance phases of control and for use on a «self-help-basis», would seem fully justified. With this perspective there is plenty of reason to discuss the characteristics and value of plant molluscicides and in this framework the integrated efforts to assess the merits and drawbacks of *Ambrosia maritima* would seem fully justified.

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THE USE OF PLANT MOLLUSCICIDES IN SCHISTOSOMIASIS CONTROL: ADVANTAGES, PROBLEMS, PROSPECTS

BY

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SUMMARY. — The development of plant molluscicides has been overshadowed in the past by the advantages which seemed to be offered by commercially available products. More recently, changes in emphasis in schistosomiasis control strategy with the development of chemotherapy and the use of integrated disease management have influenced the importance attached to intermediate host control. The need for more self-reliance in affected communities and cost-effective health care delivery systems have caused a renewed interest in plant molluscicides. Although they do kill snails at dosages which, in some cases, make them a practical proposition for field use, their sustained application through community participation is not yet proved. In the particular case of *Ambrosia*, variation in field activity between Egypt and Senegal requires a convincing explanation.

RÉSUMÉ. — *L'emploi des molluscicides d'origine végétale dans la lutte contre la schistosomiase : Avantages, problèmes, perspectives.* — La mise au point de molluscicides d'origine végétale a été masquée dans le passé par les avantages que paraissaient présenter les produits disponibles sur le marché. Plus récemment, les changements dans la considération d'une stratégie de lutte contre la schistosomiase à la suite du développement de la chimiothérapie et de la mise en œuvre d'une approche intégrée de la maladie, ont influencé l'importance accordée à la lutte contre l'hôte intermédiaire. La nécessité d'une autonomie accrue des populations affectées et de systèmes de dispensation des soins moins coûteux a renouvelé l'intérêt pour les molluscicides d'origine végétale. Bien qu'ils tuent les mollusques à des doses qui, dans certains cas, permettent de proposer leur emploi sur le terrain, leur application durable par la participation de la population n'a pas été prouvée. Dans le cas particulier d'*Ambrosia*, les différences de l'activité sur le terrain entre l'Egypte et le Sénégal attendent encore une explication convaincante.

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SAMENVATTING. — *Het gebruik van plantenmollusciciden in de bestrijding van schistosomiasis: Voordelen, problemen, perspektieven.* — Het gebruik van plantenmollusciciden werd in het verleden overschaduwd door de voordelen die de commercieel beschikbare produkten schenen aan te bieden. Recenter werd ook het belang in de bestrijding van de intermediaire gastheer beïnvloed door de wijzigingen in de visie van een bestrijdingsstrategie tegen de schistosomiasis, ten gevolge van de ontwikkeling van de chemotherapie en van de ontwikkeling van een geïntegreerde benadering van de ziekte. De noodzaak van een grotere zelfstandigheid van de aangetaste gemeenschappen en van goedkopere systemen van zorgdispensatie heeft de belangstelling voor de plantenmollusciciden hernieuwd. Ondanks het feit dat zij de slakken doden bij dosissen die ons in sommige gevallen toelaten hun veldgebruik voor te stellen, werd hun duurzame toepassing door de deelname van de bevolking niet bewezen. In het bijzonder geval van *Ambrosia* vereist het activiteitsverschil op het terrein tussen Egypte en Senegal nog een overtuigende verklaring.

Introduction

The molluscicidal activity of plants has been known for some time (MOZLEY 1952). Schistosomiasis intermediate host control has however been dominated by commercially available products which can be produced in quantity to a high specification and formulated for specific applications. We have seen, however, growing disenchantment with chemical molluscicides with increased concern over effects on non-target organisms and the environment in general and the fact that their use rarely seemed to lead to anything much more than a transient control of the snail and transmission. In addition, the relatively small size of the molluscicide market has not encouraged chemical companies to invest heavily in R & D to produce new and better products. On the other hand, there has more recently been much effort put into chemotherapy where advances have certainly been made and into immunology and the promise held out by vaccines. But, still prevalence of the disease persists and many workers, in common with colleagues in other areas of pest and disease control, now believe that an integrated strategy for disease management is the best way forward. In the meantime, the cost of chemicals has increased and their purchase eats into the much needed capital of those public health workers in the countries affected by the disease.

There has therefore been a reconsideration of plant molluscicides as an alternative snail control measure (MOTT 1987). For this purpose, they should be common in the endemic areas of concern, available at or near transmission sites at the right season or be storable at least, either cheap and easy to cultivate or gather from the wild and so, represent an economic means of snail control. There has also been much talk of the benefits from the participation of local communities in the use of plant molluscicides; benefits in improved knowledge and recognition of diseases in general, self-support and independent action.

Achievements to date

A great deal has certainly been accomplished. Many plants have been bioassayed, field trials have been conducted, evidence of acute and chronic toxicity collected, active ingredients identified and quantified and agronomic and taxonomic studies carried out. Work on *Phytolacca* and *Ambrosia* has been going on for some thirty years. But, there has not been, to the author's knowledge, any practical, self-sustaining programme for the use of plant molluscicides anywhere.

Snail and transmission control

Work in Adwa, Ethiopia showed that treatment of two small rivers in the town with slurries of dried, ground berries of *Phytolacca* gave a marked reduction in *Biomphalaria* snail numbers and prevalence of *Schistosoma mansoni* (LEMMA 1970). In the case of *Ambrosia*, a series of field trials in the Nile Delta indicated that a single, correctly-timed application of either fresh or dry plant would again reduce snail numbers to low levels (EL SAWY *et al.* 1983, 1984, 1987).

The Nile Delta is one of the hyperendemic areas for schistosomiasis. There are innumerable irrigation canals and drains and almost daily water contact for the farmers and their families. Prevalence of *S. mansoni* is high. The transmission season begins in May and ends in September-October. The snail breeding season is virtually over by May. The plant is an annual, with a tendency to perennate, a preferred habitat on watercourse banks and attains its full vegetative growth by May. The plant is less common in the Delta than previously for reasons which are not known. However, it can be cultivated in field plots and on irrigation watercourse banks.

From a consideration of the seasonal relationships of transmission season, snail breeding and plant growth, it was thought that if the plant could be grown on irrigation watercourse banks and cut and thrown into the water in May, the snail population would not be likely to recover until the following year and that this could represent a very effective control system.

Transmission control has not yet been demonstrated for *Ambrosia*. This is in spite of the fact that snails carrying cercariae have not been found after plant molluscicide application at the village level. It may be that the usual snail sampling techniques employed are not sensitive enough and more diligent approaches are called for. Egyptian villagers do move about outside their immediate village neighbourhood and may be picking up the infection outside the treated village areas. Treatment on a much larger scale would be required to counteract mobility.

Community participation

In further developing a delivery system for *Ambrosia*, it was reasoned, since farmers usually work only an acre or two of land each and this land is bordered by irrigation channels, farmers or villagers could be given seed and asked to plant on watercourse banks in November. They might then have to do some weeding and watering until the young plants became established in the next month or so and harvest in May, simply throwing the plants into the adjacent water while retaining some for seed for the following year. This should not take up more than three or four days per year altogether which is presumably not a very large investment towards their own well-being. With some organization — most villagers have television and radio — planting and harvesting could be regionally co-ordinated and result in large areas, if not the whole Delta, being treated at the same time of year. One reason why chemical molluscicides have not been successful in the Delta is that their application has relied on a relatively small number of government spray teams who simply could not cover enough ground at the right time of year.

The one aspect which had not been budgeted for accurately was the willing collaboration of the local population. They have not to date shown a great interest in running such a programme for themselves. A KAP (Knowledge, Attitudes and Practice) study has been conducted to find out what factors would influence community involvement but this has not been reported on as yet. Although the population of Adwa was involved in river clearance and application of *Phytolacca*, recurrent treatment was mainly the responsibility of an outside team of researchers. It may turn out, therefore, that motivation and sense of community are merely western values which have no real meaning in African or Middle Eastern cultures. In which case, all plant molluscicide delivery through the local community is doomed to failure.

DUNCAN & STURROCK (1987) recommended a feasibility study of the use of a plant molluscicide before beginning any practical work. Such a study should include information on whether local people are likely to become involved and also whether local and central health authorities will provide funding or infrastructural support to the larger scale employment of a plant molluscicide methodology in schistosomiasis control.

Intraspecific variability in plant molluscicidal activity

Field dosages of 70 and 35 ppm dry *Ambrosia* have been reported as lowering snail numbers in Egypt. It should be added that a dosage level which does not work has not yet been reported. On the other hand, results from Senegal suggest that much higher dosages have to be employed and even these may not work at all under certain conditions. Various reasons have been put forward for these differing results; species of snail, variety of plant, depths of water and temperature (GEERTS *et al.* 1991). In Egypt at least, *Ambrosia* seems to be unique among molluscicides in being more active in the field than in the laboratory and a convincing explanation is required for this. Most studies appear to have assumed that the active principles work by contact action and that the delayed molluscicidal effect, which seems to happen in both of the above countries, is due primarily to the low water solubility of sesquiterpene lactones. Another possibility is that the toxic action is chronic, activity appears to be delayed in both Senegal and Egypt, and this may be the result of the active ingredients being absorbed onto suspended mud and becoming attached and ingested along with the periphyton which snails browse upon. This process may be affected overall by temperature.

There is a case therefore for re-examining the available data relating to mode of action and in relation to the physicochemical properties of the active ingredients. Hopefully, it may be possible to simulate levels of field activity in the laboratory so that the reasons for the intra-country effects of the plant might be elucidated.

Conclusion

Although plant molluscicides are, on the face of it, a solution to the problem of snail control which could be taken up by many of those countries afflicted with schistosomiasis, it is evident that, for a number of reasons, their day is not yet come and it will require a determined and objective approach to overcome some of the barriers discussed above.

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USEFULNESS OF *PHYTOLACCA DODECANDRA* BERRIES FOR CONTROL OF SNAIL POPULATIONS

BY

CH. B. LUGT *

SUMMARY. — Agro-chemical selection issues regarding molluscicidal potency and pest resistance of *Phytolacca dodecandra* types are described. *P. dodecandra* types were selected with 100% snail-killing potency after 24 h of exposure at 10 ppm concentrations and their berries used in field trials. A method of application by siphoning a *P. dodecandra* berry suspension into running water of a river during the dry season in Bati, Northern Ethiopia, is described. A river, densely populated with *Biomphalaria pfeifferi* and other snail species, was used for test treatments with only a few kgs of berries per treatment. The presence of snails could be reduced to almost nil. Except for fish, the water fauna in the stretch of river treated and monitored was not affected. Fish was present up-stream, so that they could infiltrate the treated part as soon as the molluscicide had disappeared.

RÉSUMÉ. — *L'utilité des baies de* Phytolacca dodecandra *pour la lutte contre les populations de gastéropodes.* — La sélection de types de *Phytolacca dodecandra* sur des bases phytotechniques et chimiques en ce qui concerne leur pouvoir molluscicide et leur résistance aux maladies est décrite. Des types sélectionnés de *P. dodecandra*, dont les baies ont, à la concentration de 10 ppm, après un contact de 24 heures avec les gastéropodes, un pouvoir molluscicide de 100%, ont été utilisés au cours de tests sur le terrain. Une méthode d'application par siphonage lent d'une suspension de baies de *P. dodecandra*, en saison sèche, dans l'eau courante d'une petite rivière à Bati, dans le nord de l'Ethiopie, est décrite. La rivière, peuplée de millions d'individus de *Biomphalaria pfeifferi* et d'autres gastéropodes, a été traitée au moyen de seulement quelques kilogrammes de poudre de baies. La présence des gastéropodes a ainsi pu être pratiquement réduite à zéro. À l'exception des poissons, la faune aquatique du bief traité et suivi n'a pas été sensiblement affectée. En amont du point de traitement, les poissons étaient toujours présents de sorte qu'après disparition du molluscicide, ils pouvaient recoloniser la section de la rivière qui avait été traitée.

SAMENVATTING. — Landbouwkundige en chemische selectie betreffende ziekteresistentie en slakkendodend vermogen van *Phytolacca dodecandra* wordt beschreven.

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Geselecteerde *P. dodecandra* typen, waarvan de bessen na 24 uur contact met slakken bij een 10 ppm concentratie 100% doding veroorzaken, worden gebruikt in veldproeven. Een methode, waarbij een *P. dodecandra* bessen suspensie middels een siphonsysteem langzaam aan een stromend riviertje in Bati, Noord Ethiopië, wordt toegediend tijdens het droge seizoen, wordt beschreven. De rivier, bevolkt met miljoenen *Biomphalaria pfeifferi* en andere slakkensoorten, wordt experimenteel behandeld met slechts enkele kilogrammen bessenpoeder. De aanwezigheid van deze slakken kon bijna tot nul gereduceerd worden. Behalve vis, werd de fauna in het behandelde deel van de rivier niet merkbaar aangetast. Bovenstrooms van het toedieningspunt bleef vis aanwezig en kon, na verdwijning van de *P. dodecandra* bessen suspensie, weer het behandelde deel van de rivier bevolken.

1. Introduction

The information on usefulness of *Phytolacca dodecandra* berries (Fig. 1) to control snail populations as presented here, was collected in Ethiopia during a period of more than 4.5 years and ended in the summer of 1981.

During the sixties and early seventies molluscicidal activity of *P. dodecandra* berries, or Endod as it is called in Ethiopia, was reported by LEMMA (1970) and co-workers. After my departure from Ethiopia, the project continued there and transfer of high quality plant material (cuttings and seeds) took place to other East African countries. Of my selections small plantations have been set out in Malawi, Swaziland, Zambia, Tanzania, possibly in Zimbabwe.

In the following chapters, I will guide you through the essential parts of the bulk of the information collected in respect to agrobotanical issues, the processing of berries prior to application, the actual application, and I will present you with an assessment of the usefulness of synthetic and other plant molluscicides *vis a vis* the berries of *P. dodecandra*.

2. Occurrence, botany and agriculture

P. dodecandra l'Hérit. (Fig. 1) occurs naturally along roads, in fences and in forest clearings at altitudes of 1,000 m and higher in Central and East Africa. The plant produces so-called racemes on which berries are located. Racemes can measure up to 40 cm. Only in Ethiopia the berries are used as a natural soap, and it is therefore that the plant can be found near villages and streams. The kind of berries, sold on the markets to launder clothes, are fully developed unripe

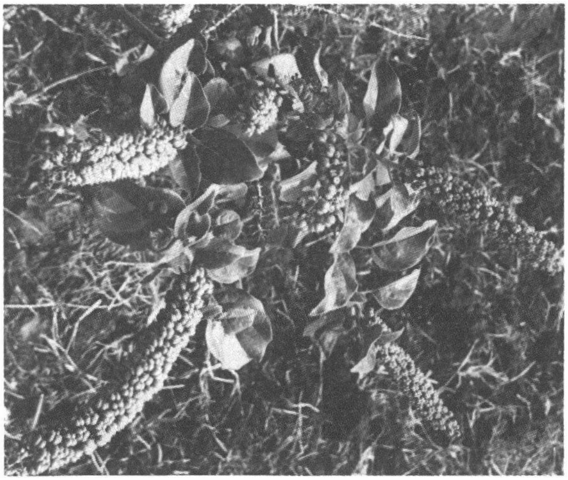

Fig. 1. — Berries on female racemes of *Phytolacca dodecandra*.

berries. Later, laboratory experiments revealed that this unripe stage is the best stage for the berries to be used for snail-killing purposes (LUGT 1980).

As from 1976 on, an active agro-chemical research, based on chemical (high molluscicidal) selection and on pest resistant quality, was added to the work already done earlier.

P. dodecandra occurs in a wide morphological variation: there are reddish and green types, hairy and smooth types (LUGT 1989). Differences also occur in respect to shape and size of leaves and in the habitus of whole plants. Following the selection of high performing types and because of the possibility of cross-pollination, plantations were set up consisting of clones propagated through cuttings. The reason was that there are so-called male and female plants, with only female plants producing berries. It appeared, however, that berry production was not restricted when male plants were not present. Self-pollination takes place through insects, like ants and small flies.

Propagation through cuttings can be highly successful with rooting percentages of up to 90%. After 5 to 6 weeks the rooted cuttings can be planted out on their final place, while these plants start to produce berries half a year later. With good care the plants, or rather bushes, can survive a long period of time.

The wild *P. dodecandra* may have a problem with Drosophilid types of insects (*Gitona* spp.), whose larvae mine stems and leaves and can cause considerable damage to plants. It was discovered, however, that plant types with hairs on leaves and stems and those with bundles of needle shaped crystals in the mesophyll leave tissue could resist and overcome these attacks. Selection efforts were thus focussed on plants with these two properties. For the rest there were no serious phytosanitary problems.

In order to secure a sufficient berry production, the plants were trimmed once a year during the rainy season. If the bushes were planted at a space of 1 × 2 m, individual bushes produced 250 g of dried berries per year.

In the light of the kilogrammes necessary for snail treatment under the circumstances to be described later, these figures give hopeful perspectives for self-help control purposes of snails in infected waterbodies on the local village level.

3. Processing of berries

Berries can be collected over a relatively long period of time; in Ethiopia from November until May. This means that berry picking has no constraints in respect to peak activities. Only the fully grown unripe racemes with berries are taken and dried in the shade. A moisture content of not more than 8% will secure its molluscicidal activity for years.

The dried berries as such are non-molluscicidal, which was shown by the fact that an alcohol extract of powdered dried berries had no molluscicidal activity.

However, in contrast, when powdered dried berries were mixed with water and kept for some time to rest in the water, molluscicidal activity emerged (through enzymatic cleavage of saponin-sugar bonds). One can see on TLC that Rf values of individual saponin spots increase compared to the spots of an ethanolic extract of the same origin of berries. In the presence of water the saponins in the berries become molluscicidal.

4. Application issues

For the test-applications in the field, the berries were ground to a fineness of 0.5 mm and smaller.

Disadvantage of this fine powder was that it often provoked allergic reactions, so that the person handling the powder had to take precautions to avoid inhalation of dust. For routine practices it is therefore probably wise to grind the berries not too small and to use a little more berries than calculated as being necessary for river treatment.

On the basis of experiences from the past (LUGT 1981, 1982), for future applications, all the different essential steps are presented hereafter in the form of check lists.

4.1. KILOGRAMMES OF BERRY POWDER NECESSARY FOR FOCAL RIVER TREATMENT

a. Application must be carried out in the dry season, so that less water has to be treated while snails are confined to (concentrated in) a small volume of water.
b. Of the river to be treated an inventory has to be made as to the occurrence of snails, other organisms, weeds, ponds, cataracts, etc. The water body to be treated has to be thoroughly mapped (Fig. 2).
c. It is then decided where the application will be located; in any case up-stream from the last place of occurrence of snails and preferably in a narrow turbulent part of the river, allowing a complete mixture of the berry suspension with the river water.
d. In the laboratory, the berry powder to be used has to be tested on the lowest ppm concentration giving 100% snail-kill after 5 hours of exposure.
e. For efficacy reasons under natural river conditions, the ppm conc. giving a 100% snail-kill in the laboratory has to be raised by half.
f. The water discharge is calculated and if necessary reduced by diverting water up-stream. One should try to arrive at a semi-stagnant water situation.
g. Having calculated the water discharge as well as the desired final ppm berry conc. for river conditions (*vide* e. and f.), the suspension must be siphoned to the river with such a speed that over a period of 5-6 hours a «cloud» with the desired molluscicidal strength moves slowly down-stream to cause optimal snail-molluscicide contact over a period of at least 5 hours (Fig. 3).

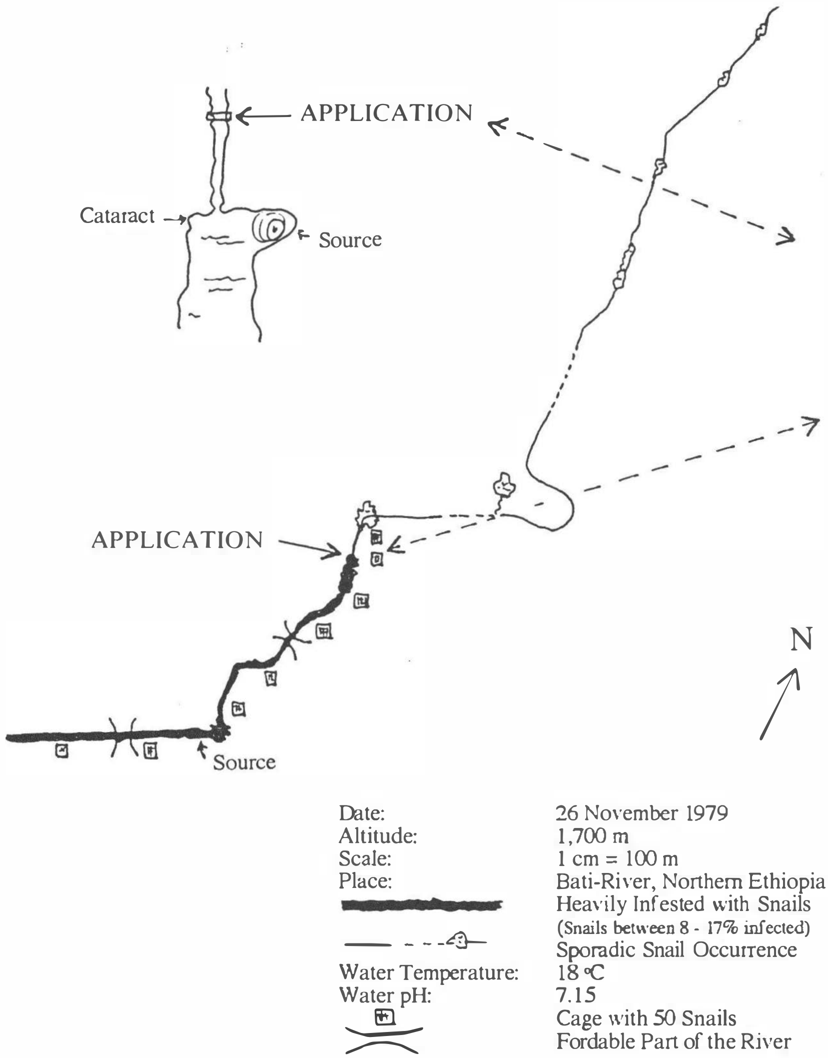

Fig. 2. — Focal application of a *Phytolacca dodecandra* berry suspension in Bati river (Ethiopia). The river is mapped while snail densities, cages and water discharges are indicated.

Under the circumstances described earlier (LUGT 1981, 1982), the quantity of berry powder, applied during these experiments, was not raised by half. For these case studies the quantities of berry powder used varied between 2.25 and 3.5 kg.

Fig. 3. — Siphoning of the berry suspension into the river.

Referring to what was mentioned earlier, we see that such quantities can be the yield of 20 *P. dodecandra* bushes and coming from 40 m^2 of land, which should not pose a big problem for health centres in parts of Ethiopia and must be largely sufficient for treatment of a river like the one passing Bati: a river used by tens of thousands of people and infested with millions of *Biomphalaria pfeifferi* snails, which were on some spots for 17% infected with the *Schistosoma mansoni* parasite.

4.2. Preparation, organization and execution of the molluscicide application

a. A few days before treatment, local authorities must be informed on the purpose and the extent of the experiment. If necessary help must be secured to guard the stretch of the river to be monitored.
b. Before application the stretch of river to be treated must be mapped and an inventory be made regarding biological data (number and kind of snail species, insect species, tadpoles, etc.).
c. On the day of application the barrel with the suspension has to be installed as early in the morning as possible and the berry suspension released in mls of suspension per minute on the basis of litres of river water passing the application point per minute.

d. In the meantime, snails have to be collected down-stream for monitoring the molluscicide's efficacy. Then snail cages are prepared and well hidden in the river stretch to be monitored, while a number of snails are kept for a check on the water samples later.
e. Every 5-10 minutes the flow of the suspension ($ml.min^{-1}$) has to be checked.
f. If application started around 7.00 hours, after a well controlled release of the suspension, at around 13.00-14.00 hours no liquid will be left in the barrel. The deposit in the barrel may be released in the river in order to provide for an additional snail-kill effect.

4.3. Monitoring the effect

This can be carried out in 3 different ways:

a. Snails are collected before molluscicide application starts and iron mesh-wire cages are prepared with 50 snails. Hereafter these cages are placed at regular intervals and must be well hidden in the river (Fig. 4).
b. Water samples must be taken at regular time intervals and on different places in the river, followed by a check on snail-kill potency.
c. Scooping snails to assess the impact of the molluscicide on the free living snails is difficult but it may provide for essential additional information. One has to develop a standard method (size of scoop, number of scoops, mesh wire width, etc.) and repeat this method systematically along the river.

During and after application dead snails drop on the bottom of the river and it may sometimes be difficult to recover them.

It was, however, undisputable that after application, millions of floating snails had disappeared and that one was able to conclude that a majority of the snails must have been killed as a result of treatment of the river with the *P. dodecandra* berry suspension.

4.4. What one will observe during and after treatment

a. Soon after application has started, foam will develop in the river. More down-stream, one will observe that fish behave as alarmed, thus indicating that the molluscicide is near. Nearer to the place

Fig. 4. — Recovery of a well hidden snail cage.

of application, one will see trapped fish dying. Fish is a good indicator of the actual movement of this molluscicide.

b. The cages will indicate the effectiveness of the treatment, while water samples will indicate when and for how long the treatment was effective. These results will be backed up by results coming from systematic scooping of snails in the river.

Observations case reports (Lugt 1981, 1982)

c. Regarding other organisms, the day after treatment, we observed the presence of tadpoles, dragon-flies, water-beetles, while the water was extremely clear.

d. On the places where snails were present the day before, apart from a few snails, not the massive presence of millions of snails could be observed. What was observed was that snail eggs had survived the treatment.
e. Of the fish: *Discognathus (Garra) blanfordii*, all of them had disappeared. But up-stream the same fish was waiting to enter the stretch of river treated as soon as the situation would be safe for them.

4.5. Conclusions

Although our case studies had a clearly experimental character, one could conclude that this method of application had a tremendous impact on the snail population, while for the rest of the environment no visual negative consequences could be observed. In fact it looked as if this type of treatment had just taken out the type of organisms for which the experiment was designed.

It is therefore that the effectiveness of *P. dodecandra* berries as an effective molluscicide, at relatively small quantities of berry material, cannot be disputed.

5. A comparison: Synthetic and plant products

While comparing plant molluscicides, one can only do so when information on the following four parameters is available:

a. Molluscicidal potency expressed in ppm of the absolute dry material;
b. Solubility and miscibility in water of the dried material (a.);
c. Yield per square area of the dried plant molluscicide material (a.);
d. Feasibility of cropping and availability on a regular basis (of a.).

Table 1 gives information regarding a. and b. only. With respect to items c. and d., one can say for *P. dodecandra* that 1,250 kilogrammes can be harvested from one hectare (10,000 m^2) and that the plant (bush) is a perennial so that regular production can be secured. Most probably, the same applies to *P. octandra*, but it is of interest to see what capacities the other plant species have.

Table 1

100% snail-kill in relation to concentration and usefulness with respect to solubility, resp. miscibility in water

Molluscicide (Origin)	100% snail-kill at ... ppm	Solubility/Miscibility in water
Frescon® 16.5% a.i. (Shell Ethiopia)	2.34	good
Bayluscid® (Bayer Ethiopia)	0.32 - 1.25	poor
Phytolacca dodecandra type 35 H_2O extr. (Ethiopia)	6.25	good
P. dodecandra type 4 berries (Ethiopia)	15	good
P. dodecandra type 17 berries (Ethiopia)	10	good
P. dodecandra type 44 berries (Ethiopia)	10	good
P. octandra unripe berries (Dr. Ouma, Kenya)	> 50	good
P. octandra ripe berries (Dr. Ouma, Kenya)	100	good
Polygonum senegalense (Dr. Ouma, Kenya)	none	difficult
Croton macrostachys (Dr. Amin, Sudan)	none	difficult/impossible
Sesbania sesban (Ethiopia)	100	difficult
Ambrosia maritima (Dr. El Sawy, Egypt)	> 200	difficult

Studies in the laboratory and visualized in Table 1 were carried out with *Biomphalaria glabrata* snails and under equal conditions.

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SWARTZIA MADAGASCARIENSIS, TETRAPLEURA TETRAPTERA, AND ANALYTICAL ASPECTS OF PLANT MOLLUSCICIDES

BY

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SUMMARY. — *Swartzia madagascariensis* (Leguminosae) and *Tetrapleura tetraptera* (Leguminosae) are two plants which have shown important molluscicidal activities in laboratory experiments. Since many of the criteria for plant molluscicides are fulfilled by both species, they were selected for limited trials in the field (in Tanzania, for *S. madagascariensis*, and in Nigeria for *T. tetrapleura*). Fruit extracts of the two trees were found to contain a high concentration of molluscicidal saponins. Some of these compounds are among the most powerful natural molluscicides and have similar potencies to synthetic molluscicides. Field trials confirmed that the molluscicidal activity observed in the laboratory remained unchanged when applied in a natural habitat, i.e. when exposed to physicochemical influences. A brief outline of these trials is given, together with some considerations about general toxicity. In the second part, the analysis of active principles in natural plant molluscicides is described. This is illustrated by quantitative and qualitative HPLC analyses of saponins from different plants, including *Phytolacca dodecandra* (Phytolaccaceae), and of sesquiterpene lactones from *Ambrosia maritima* (Asteraceae).

RÉSUMÉ. — Swartzia madagascariensis, Tetrapleura tetraptera *et analyse de molluscicides naturels.* — *Swartzia madagascariensis* (Leguminosae) et *Tetrapleura tetraptera* (Leguminosae) sont deux plantes dotées d'importantes activités molluscicides. Comme elles répondent à certains critères prédéfinis, elles ont été sélectionnées pour des études pratiques sur le terrain (en Tanzanie avec *S. madagascariensis*, au Nigéria pour *T. tetrapleura*). Les extraits des fruits de ces deux espèces contiennent des saponines molluscicides en concentration importante. En particulier, certains de ces composés s'avèrent être parmi les plus puissants molluscicides naturels, et possèdent des activités comparables à celles des produits de synthèse. Les essais pratiques ont confirmé les potentialités de ces deux plantes dans le cadre de la lutte contre la

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schistosomiase. Un rapide aperçu de ces expériences est présenté, ainsi que quelques considérations sur la toxicité générale des extraits de ces plantes. Dans la deuxième partie, l'analyse de principes actifs de plantes molluscicides est décrite. Ces techniques sont illustrées par différentes analyses qualitatives et quantitatives par CLHP des saponines de quelques plantes molluscicides, en particulier *Phytolacca dodecandra* (Phytolaccaceae). La détermination quantitative des lactones sesquiterpéniques de *Ambrosia maritima* (Asteraceae) est également présentée.

SAMENVATTING. — Swartzia madagascariensis, Tetrapleura tetraptera *en analyse van natuurlijke molluscicіden.* — *Swartzia madagascariensis* (Leguminosae) en *Tetrapleura tetraptera* (Leguminosae) zijn twee planten welke aanzienlijke molluscicische activiteit vertonen. Aangezien beide planten voldoen aan de gestelde eisen (voor natuurlijke molluscicіden), zijn ze geselecteerd voor veldstudies (in Tanzania met *S. madagascariensis*, in Nigeria met *T. tetraptera*). Vruchtextracten van beide bomen bleken rijk aan molluscicidal saponienen. Enkele van deze verbindingen vallen onder de meest effectieve natuurlijke molluscicіden, en hebben activiteiten vergelijkbaar met die van synthetische molluscicіden. Veldstudies bevestigden dat de molluscicische activiteit, gevonden in laboratoria, niet afweek wanneer aangewend in de natuurlijke habitat, i.e. blootgesteld aan fysicochemische invloeden. Een korte beschrijving van deze studies wordt hier gepresenteerd, samen met enkele overwegingen betreffende de algemene toxiciteit van deze extracten. In het tweede deel wordt de analyse van de actieve bestanddelen van natuurlijke molluscicіden beschreven, geïllustreerd door kwantitatieve HPLC-analyses van saponienen van verschillende planten, waaronder *Phytolacca dodecandra* (Phytolaccaceae), en sesquiterpene-lactonen van *Ambrosia maritima* (Asteraceae).

Introduction

Molluscicides represent a crucial factor in an integrated approach to the control of schistosomiasis. At the present time, Bayluscide (2,5′-dichloro-4′-nitrosalicylanilide or niclosamide) is the only molluscicide recommended by the World Health Organization (WHO). However, expensive synthetic compounds pose a problem in anti-bilharzia programs, when the deciding factor, especially for Third World countries, is the cost. Synthetic molluscicides, like Bayluscide, in addition, may result in problems of toxicity to non-target organisms and deleterious long-term effects on the environment. The possible development of resistance in schistosomiasis-transmitting snails is another important factor. In contrast, the use of plants with molluscicidal properties is a simple, inexpensive and appropriate technology for local control of the snail vector (MARSTON & HOSTETTMANN 1985).

Furthermore, investigation of plants used in traditional medicine or recorded in ethnopharmacological literature provides a ready means of increasing the diversity of available molluscicides and simplifying the choice of selective, ecologically safe snail-killing compounds (FARNSWORTH *et al.* 1987).

Since the 1983 WHO-organized meeting of the Scientific Working Group on Plant Molluscicides held in Geneva, the number of reports on the use of plant molluscicides has been augmented considerably (HOSTETTMANN & MARSTON 1987).

More than 1,000 plant species have been tested for molluscicidal activity (KLOOS & MCCULLOUGH 1982). However, studies on long-term toxicity against non-target organisms (including man) and observations on the mutagenicity/carcinogenicity of these plant molluscicides are rare (MCCULLOUGH & MOTT 1983).

In addition, only a small proportion of the numerous plants showing *in vitro* molluscicidal activity are liable to represent a good alternative in the field control of the vector of the parasitic disease schistosomiasis. This is because there are several prerequisites for a viable candidate plant molluscicide (MARSTON & HOSTETTMANN 1985, HOSTETTMANN 1989):

a) The molluscicidal activity should be high. The crude extract from which the compounds are obtained should have an activity at concentrations lower than 100 mgL^{-1}. The activity of the strongest synthetic molluscicides lies around 1 mgL^{-1}; e.g. for trifenmorph, the LC_{100} against the schistosomiasis-transmitting snail *Biomphalaria glabrata* is 0.25 mgL^{-1} after an exposure time of 24 h. Similar values are obtained for *Bulinus* snails. Consequently, to be effective competitors in regard to synthetic molluscicides, plant molluscicides must have LC_{100} values of this order of magnitude. It is advantageous if the molluscicide also kills snail eggs.
b) The plant in question should grow abundantly in the natural endemic area. Either the plant should be of high natural abundance, or alternatively, easily cultivated. In addition, regenerating plant parts (fruits, leaves or flowers) should be used and, if possible, not the roots, since this leads to the destruction of the plant.
c) Extraction of the active constituents by water is an advantage. The cost of organic solvents and accompanying extraction apparatus could be prohibitive for schistosomiasis control programmes in Third World countries.

d) Application procedures should be simple and safe to the operator. In addition, formulations and storage must be straightforward.
e) The plant extract or molluscicide should possess low toxicity to non-target organisms (including man). The discovery of compounds more specifically toxic to snails would be a great advantage. Furthermore, isolation of the active compounds from those molluscicidal plants which are potentially applicable to field trials is important for toxicological and environmental studies.
f) Costs should be low.

Regarding these different criteria of selection, it is obvious that the probability of finding a unique molluscicidal plant which can be used in all endemic areas is very small. Judicious solutions have to be established from case to case, according to the native flora, and the local situation. Thus, for example, among the different promising natural molluscicides, Endod (*Phytolacca dodecandra*, Phytolaccaceae) has been intensively studied. The fruits of this plant contain triterpenoid saponins with high molluscicidal activities (PARKHURST *et al.* 1973, DOMON & HOSTETTMANN 1984, reviewed by MARSTON & HOSTETTMANN 1985 and DORSAZ & HOSTETTMANN 1986) and promising field trials to control schistosomiasis have been undertaken with Endod in Ethiopia (GOLL *et al.* 1983). Nevertheless, the geographical distribution of *Phytolacca dodecandra* is restricted to Ethiopia, and thus it has to be cultivated if required for use in other countries. Therefore, additional plants have to be taken into account.

Other field trials have been performed with *Ambrosia maritima* (Asteraceae) in Egypt (EL SAWY *et al.* 1984) and with *Anacardium occidentalis* (Anacardiaceae) in Mozambique (WEBBE & LAMBERT 1983). Once again, however, these different plants, which exhibit promising molluscicidal activities show restricted geographical distribution patterns. Some of them are not necessarily abundant in the areas where schistosomiasis is endemic. In addition, there are complications arising from the allergenicity of constituents, particularly in the case of *A. occidentalis.* This emphasizes the need to search for other plant molluscicides which will meet the prerequisites defined above.

1. *Swartzia madagascariensis* and *Tetrapleura tetraptera*

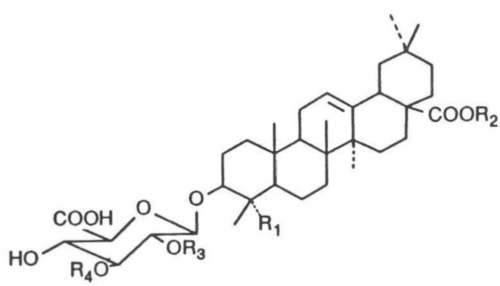
1.1. *Swartzia madagascariensis*

Among the different plants in Africa with molluscicidal activity (Haerdi 1964, Farnsworth *et al.* 1987, Kloos & McCullough 1987), *Swartzia madagascariensis* (Leguminosae-Caesalpinioideae) was selected for one of our investigations.

This tree is very common in many regions of Africa. Since 1939, there have been reports that fruits of this plant have been used effectively in controlling the populations of schistosomiasis-transmitting snails in natural ponds (Mozley 1939, Mozley 1952).

Large quantities (up to 40 kg) of the inedible fruits can be obtained from each tree, and 100 mgL^{-1} of dry seed pods in water kills over 90% of *Bulinus globosus* snails exposed for 24 hours to the molluscicidal extract. As many of the criteria for plant molluscicides are fulfilled by *S. madagascariensis*, it was selected for limited trials in the field (Suter *et al.* 1986).

Phytochemical investigation showed that molluscicidal activity was linked to the saponin content of this plant. Five compounds,



Saponin[a)]	R_1	R_2	R_3	R_4	Molluscicidal activity[b)]
1	CH_3	H	H	Rha	3
2	CHO	H	H	Rha	25
3	CH_3	H	Glc	Rha	25
4	CHO	H	Glc	Rha	> 50
5	CH_3	Glc	Glc	Rha	no activity

a) Glc = β-D-Glucopyranosyl, Rha = α-L-Rhamnopyranosyl

b) Molluscicidal activity against *Biomphalaria glabrata* [mgL^{-1}].

saponins **1** - **5**, were chromatographically isolated from the molluscicidal aqueous extract of the dried ground pods (BOREL & HOSTETTMANN 1987).

These compounds were shown to be glucuronides of oleanolic acid and gypsogenin. Saponins **2** - **5** had not previously been isolated, whereas saponin **1** is a known compound found in other plants (BOREL & HOSTETTMANN 1987).

The results of biological testing demonstrated that saponin **1** presented the highest molluscicidal activity of the isolated compounds against schistosomiasis-transmitting snails. This saponin showed a toxicity of $LC_{100} = 3\ mgL^{-1}$ after exposure to *Bulinus globosus* and *Biomphalaria glabrata* for 24 h. This is within the range of the molluscicidal activity of Endod (*P. dodecandra)*.

Saponins with disubstituted glucuronic acid as well as those with gypsogenin as aglycone had a lower activity ($\geq$25 ppm). In accordance with general structure-activity relationships of other molluscicidal saponins (HOSTETTMANN *et al.* 1982), bidesmosidic saponin **5** had no snail-killing activity.

These previous observations led us to continue our investigations on *S. madagascariensis*, and experiments were also undertaken in order to optimize and standardize the extraction procedure.

The results of laboratory tests revealed that an extraction time of 24 h in tap water at ambient temperature was required to achieve 100% snail mortality using dilutions of $100\ mgL^{-1}$. An extraction time between 6 and 24 h gave the best results. The extraction was not improved by heating to 50 °C. Boiling for 30 min or 1 h decreased the molluscicidal activity of *S. madagascariensis* fruit extracts. There was a significant difference in molluscicidal activity between young (green) pods and mature (dark brown) seed pods. Consequently, the more active mature, sun-dried seed pods were used for all the following experiments and field trials. Ground seed pods could be stored for 1 year without loss of molluscicidal activity (SUTER *et al.* 1986).

Further investigations showed that a 24 h water extract of $100\ mgL^{-1}$ exerted a high molluscicidal activity (snail mortality $>90\%$) to *B. globosus* exposed for 6, 12 or 24 h. Short exposure of *B. globosus* led to high mortality only at elevated concentrations of *S. madagascariensis* extracts ($\geq 400\ mgL^{-1}$) (SUTER *et al.* 1986).

It was found that an initial concentration maximum of $200\ gL^{-1}$ of ground pods could be used in order to obtain 100% snail mortality at an application level of $100\ mgL^{-1}$ after 24 h extraction.

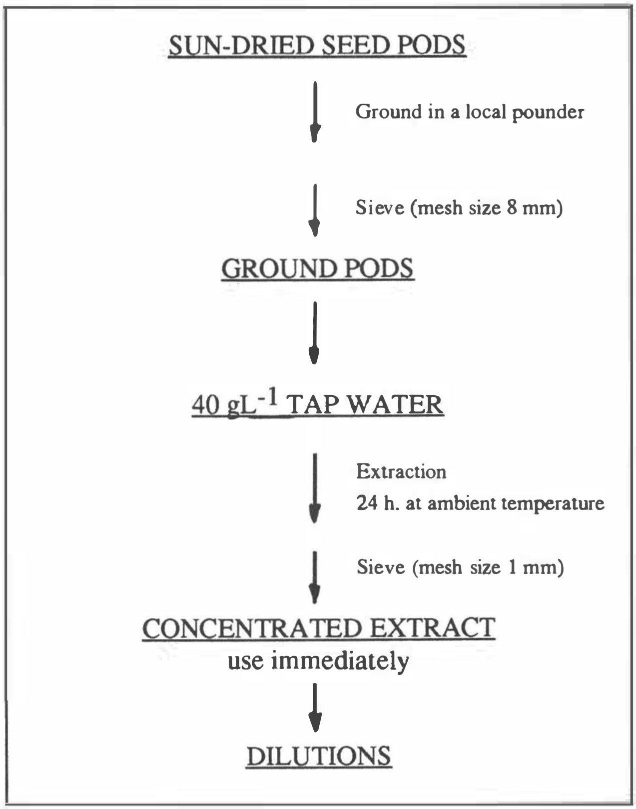


Fig. 1. — Extraction of *S. madagascariensis* fruits.

Higher initial concentrations of ground pods for extraction did not improve the molluscicidal activity of the 24 h extracts.

This first series of experiments led to the extraction procedure as outlined in Fig. 1.

As saponins are responsible for the molluscicidal activity of *S. madagascariensis* pod extract, an attempt was made to establish simple tests which would be used to measure *S. madagascariensis* saponins semi-quantitatively when applied in the field. Haemolytic capacity of treated water samples, together with mortality of snails and TLC analysis were chosen as indicators for the field trials. These methods allowed an estimation of the concentrations, as well as the degradation of *S. madagascariensis* saponins in ponds (see Table 1).

Two field trials were performed in order to test the molluscicidal activity of *S. madagascariensis* pod extracts in a natural habitat

Table 1

Field trial 1: application of S. madagascariensis *fruit extracts in a 60 m^3 pond, showing haemolysis, mortality of* Bulinus globosus *and semiquantitative determinations of saponin by thin-layer chromatography (TLC)*

	Before	Hours after application of *S. madagascariensis* extracts					
		0	6	12	24	48	72
Haemolysis[a]							
A	0	+ +	+ +	+ +	0	0	ND
B	0	+ +	+	+	0	0	ND
Mortality of snails[b]							
dead/total	0/10	50/50	50/50	50/50	43/50	14/50	6/50
TLC	0	+ +	+ +	+ +	+	0	0

[a] Haemolytic capacity of water samples collected from 5 different sites of the pond and at different times after application of *S. madagascariensis* extracts; + + = total haemolysis, + = partial haemolysis, 0 = no haemolysis, A = undiluted water samples, B = 1 : 2 diluted, each result represents the mean of the 5 sites.
[b] Mortality of encaged *Bulinus globosus*, dead/total no. of exposed.
TLC = Thin-layer chromatography of saponins; 0 = not detected, + = $\leq$50 mg ground fruitsL^{-1}, + + = > 100 mg ground fruitsL^{-1}.
ND = Not done.
Based on measurements of the water volume, *S. madagascariensis* fruit extracts were applied to reach an initial concentration of 300 mg ground fruits per liter pond water.

known to harbour *B. globosus*. These field trials were carried out at Ifakara, Tanzania, in collaboration with the Swiss Tropical Institute (Basel) and its Field Laboratory in South-Eastern Tanzania.

The area chosen for study has a high incidence of urinary schistosomiasis, with *Bulinus globosus* as the intermediate host responsible for the spread of the disease. The densities of the snails were recorded in these ponds for three years prior to the trials which were undertaken in October 1984. These data show a distinct seasonal pattern of *B. globosus* densities with a peak during the rainy season, October-February. Early October was chosen for the application of the molluscicides in these field trials as *B. globosus* densities started to increase during this month; the mollusciciding effect could thus be demonstrated by the absence of the distinct *B. globosus* density peak during the short rainy season. Furthermore, the water level in the ponds was very low at the end of the dry season.

Although laboratory experiments indicated a high molluscicidal activity of *S. madagascariensis* at 100 mgL^{-1}, higher initial concentrations were applied in the field trials in order to compensate for possible inaccuracy in water volume measurements and for the deposits of silt in the ponds.

The results of the first field trials showed that an initial molluscicide concentration of not less than 100 mgL^{-1} was reached (Table 1). Complete haemolysis was observed during the first 12 h after the application and all exposed, encaged snails died within the same period. The analysis of water samples by TLC paralleled these findings. However haemolysis was no longer observed and the mortality rates of subsequently exposed snails decreased after the application onwards.

The densities of *B. globosus* dropped to 0 one week after the single application of *S. madagascariensis* extracts. The snails were observed only at low densities and never reached the initial density during the short-term and long-term follow-up of five months. When compared with the three years before treatment, the density of *B. globosus* did not show the distinct peak between October-February and remained at very low levels.

The results of the second field trial in a larger pond (160 m^3) compared initially well with the first field trial. Haemolysis, observed up to 6 h after the single application of extract, was paralleled by the mortality of the exposed, caged *B. globosus* and by TLC (Table 2).

The density of *B. globosus* dropped to about 0, but it again reached the initial density only 8 weeks after the application. When compared to the data obtained from 1981-1983, the *B. globosus* density remained markedly lower after mollusciciding and the distinct peak October-February was no longer observed. However, the *Bulinus* population increased from January onwards, reaching densities exceeding those from previous years, and clearly indicating the need for continuing applications of molluscicide.

This study confirmed the molluscicidal properties of *S. madagascariensis* pods. The plant is a viable candidate as a molluscicide, since it meets most of the criteria proposed by the WHO (1983) and MARSTON & HOSTETTMANN (1985). Simple water extracts of sun-dried mature seed pods of this perennial tree show significant molluscicidal activity against *B. globosus*, and are easily obtained at high yields by a procedure that does not demand any sophisticated apparatus or highly trained personnel. In addition, ground fruit can

Table 2

Field trial II: application of S. madagascariensis *fruit extracts in a 160 m^3 pond, showing haemolysis, mortality of* Bulinus globosus *and semiquantitative determinations of saponin by thin-layer chromatography (TLC)*

	Before	Hours after application of *S. madagascariensis* extracts					
		0	6	12	24	48	72
Haemolysis[a]							
A	0	+ +	ND	+	0	ND	ND
B	0	+ +	ND	+	0	ND	ND
Mortality of snails[b]							
dead/total	0/10	50/50	ND	41/50	20/50	8/50	ND
TLC	0	+ +	ND	+ +	+	0	ND

[a] Haemolytic capacity of water samples collected from 5 different sites of the pond and at different times after application of *S. madagascariensis* extracts; + + = total haemolysis, + = partial haemolysis, 0 = no haemolysis, A = undiluted water samples, B = 1 : 2 diluted, each result represents the mean of the 5 sites.
[b] Mortality of encaged *Bulinus globosus*, dead/total no. of exposed.
TLC = Thin-layer chromatography of saponins; 0 = not detected, + = $\leq$50 mg ground fruitsL^{-1}, + + = > 100 mg ground fruitsL^{-1}.
ND = Not done.
Based on measurements of the water volume, *S. madagascariensis* fruit extracts were applied to reach an initial concentration of 300 mg ground fruits per liter pond water.

be stored after drying. Furthermore, *S. madagascariensis* is of abundance in the East African *Brachystegia* woodland and in many other parts of Africa. The application of pod extracts is easy and first field trials have shown the efficiency of this method against schistosomiasis-transmitting snails. However, these results also indicate that the extracts are most probably not active against *B. globosus* egg masses, as also observed for Endod (KLOOS & MCCULLOUGH 1982); this implies the need for at least one subsequent application of *S. madagascariensis* pod extract in order to achieve improved persistence of low snail densities.

The comparison of the results of our laboratory experiments with those of the two field trials indicates that the molluscicidal potency of *S. madagascariensis* pod extracts remains unchanged when applied in natural habitat, i.e. when exposed to physicochemical influences such as pH, sunlight, temperature and organic matter. Under both conditions, a half-life of 12-24 h is indicated. Obviously, this short half-life reduces the risk of toxicity to humans. The rapid biodegradability of *S. madagascariensis* is of importance since preparation and application of this plant molluscicide is appropriate for community-based action.

However, consideration must also be given to the toxicity of *S. madagascariensis* pod extracts to non-target organisms such as fish. Focal and seasonal mollusciciding schedules are likely to be the rule and thus will minimize these problems. Nevertheless, investigations on the toxicity and mutagenicity of this plant molluscicide are currently being undertaken in conjunction with the WHO.

1.2. *TETRAPLEURA TETRAPTERA*

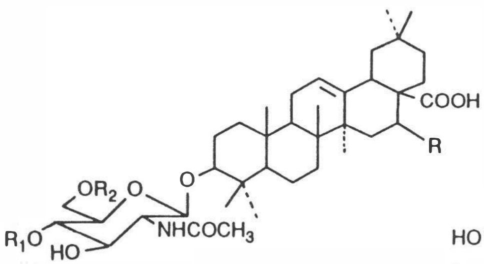
In the course of our work on molluscicidal plants, another legume has retained our attention. *Tetrapleura tetraptera* (Leguminosae-Mimosoideae), locally known as «Aridan» in the Yoruba language, is a large tree growing throughout the rainforest belt of West Africa. The plant has many traditional uses, mainly in the management of convulsions, leprosy, inflammation, and rheumatic pains (OJEWOLE & ADESINA 1983). Molluscicidal activity of this plant has been reported by several authors (ADESINA *et al.* 1980, ADEWUNMI & SOFOWORA 1980, ADEWUNMI & MARQUIS 1981). These investigators postulated that the molluscicidal activity was linked to triterpenoid saponins and coumarinic compounds.

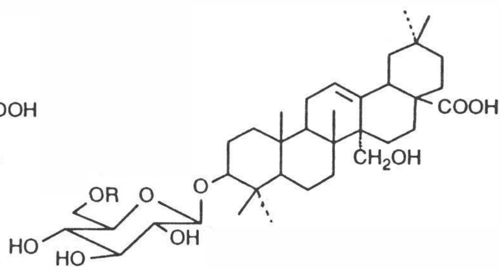
Because of the strong molluscicidal properties of the fruits of *T. tetraptera* exhibited in laboratory experiments, field trials have been carried out, and this tree is now considered to be a promising plant for the local control of schistosomiasis (HOSTETTMANN 1989).

Previous investigations of the molluscicidal extract of the fruits of *T. tetraptera* resulted in the isolation and identification of a novel oleanolic acid glycoside, aridanin **6** (ADESINA & REISCH 1985). However, it was not mentioned that this unusual mono-*N*-acetylglycoside (3-*0*-[*β*-D-glucopyranosyl-2′-acetamido-2′-deoxy]oleanolic acid) was responsible for the molluscicidal activity of the crude plant extract.

Recently, MAILLARD *et al.* (1989, 1991) undertook a reinvestigation of the plant. Four saponins (**7** - **10**), together with aridanin (**6**) could be isolated. With the exception of the inactive saponin **8**, these compounds, responsible for the molluscicidal activity of the fruits, are

N-acetylglycosides, with either oleanolic acid (6, 9, 10) or echinocystic acid (7) as aglycone. Glycosides 9 and 10 are among the most powerful natural molluscicides and have similar potencies to those isolated from *P. dodecandra* (DORSAZ & HOSTETTMANN 1986) or *S. madagascariensis* (BOREL & HOSTETTMANN 1987).





6 $R = R_1 = R_2 = H$, Aridanin
7 $R = \alpha\text{–}OH$, $R_1 = R_2 = H$
9 $R = R_1 = H$, R_2 = Gal
10 $R = H$, R_1 = Glc, $R_2 = H$

8 R = Glc

Several studies on the molluscicidal properties of *T. tetraptera* were performed before the phytochemical investigations. The first reports date from the early 1980s, when it was shown that the fruits of this tree possess remarkable molluscicidal effects (ADESINA *et al.* 1980, ADEWUNMI & SOFOWORA 1980, ADEWUNMI & MARQUIS 1981). Further studies showed that all parts of *T. tetraptera* were effective against snails (ADEWUNMI *et al.* 1982). However, the fruits seem to be the most promising part of the plant for its use in the local control of schistosomiasis.

The molluscicidal activity does not depend on the maturity of the fruit, since both green young fruits and dark-brown mature fruits are toxic to *B. globosus*.

In laboratory experiments, the fruits are toxic against schistosomiasis-transmitting snails *B. globosus*, *Biomphalaria glabrata*, *Biomphalaria pfeifferi* or *Physa waterlotti* at concentrations less than 10 ppm for the methanolic extracts. Aqueous extracts are active at concentrations less than 50 ppm (ADEWUNMI & MARQUIS 1981, ADEWUNMI 1984).

Further studies on the mode of action of the molluscicidal extracts of *T. tetraptera* have been done, and it is now known that both cellular metabolism and physiological phenomena of osmoregulation are affected in treated molluscs.

The molluscicidal activity of the fruit extracts of Aridan shows time-concentration relationships (ADEWUNMI & MARQUIS 1981), and is directly comparable to those of other molluscicidal plants (*Jatropha gossypifolia*, *Phytolacca dodecandra*, etc...) (ADEWUNMI & MARQUIS 1987). In addition, it seems that the initial activity of Aridan extracts is stronger than those from Endod (*P. dodecandra*). But, on the basis of toxicity over a 48 h exposure period, Aridan has the same molluscicidal effect as Endod. However, in habitats such as drains or swamps, where there are abrupt fluctuations of water level, a molluscicide with a rapid destructive effect is desirable. In this case, *T. tetraptera* would be superior to Endod (ADEWUNMI & MARQUIS 1987).

In field experiments, lethal concentrations of *T. tetraptera* varied between 15 and 100 ppm (ADEWUNMI *et al.* 1982, ADEWUNMI 1984), depending on the mode of extraction or the formula used. Aqueous extracts of *T. tetraptera* were capable of reducing not only the snail population in the field but were also found to keep transmission sites free from schistosome cercariae production for about 2 days (ADEWUNMI 1984). This latter activity is due to the ability of this extract to interrupt the life cycle of the schistosome at two points (ADEWUNMI & FURU 1989).

Several studies on the toxicity to non-target organisms have been carried out. The extracts of *T. tetraptera* had no noticeable effect on flora or fauna. No apparent toxic symptoms were showed in mice, birds, cats, dogs, rabbits and rats after feeding or infusing with the water extract of the fruits (ADEWUNMI *et al.* 1987).

Neither the aqueous extract of the fruit nor aridanin (**6**) induced chromosomal aberrations and sister chromatid exchanges (SCE) in Chinese hamster ovary cells, or induced mutations in the strains of *Salmonella typhii*. These results indicate that these products are not mutagenic and could be used in schistosomiasis control (ADEWUNMI 1990).

The potencies of *T. tetraptera* in the local control of the parasitic disease schistosomiasis have already been evaluated several times. During 1982, in a field control project in Nigeria, evaluation over a two-month period at various water contact sites reduced the number of *B. globosus* snails at a concentration of 10 mgL^{-1} of the methanolic extract in stagnant ponds (ADEWUNMI *et al.* 1982).

A longer study, over a two-year period, was carried out in the area of Ile-Ife (Nigeria). The water supply of 4 villages was treated every 3 months with aqueous extracts of Aridan to give a known concentration of about 20 mgL^{-1}. By this method, the density of snails was reduced by a factor of 30 during few weeks after application, and the transmission sites were kept free from cercariae for a minimum of 1 month after application of molluscicide (ADEWUNMI 1984).

More recently, some other field trials have been carried out, especially in order to determine how the application of molluscicide could be affected by the presence of weeds, oil pollution and by various physicochemical factors such as minerals in the water, pH, chemical adsorption to mud substrates, etc. Contamination with oil is responsible of the loss of activity of *T. tetraptera* extracts, and further tests should now be performed to determine the desirable level of molluscicide in each habitat, in order to have an effective molluscicidal programme (ADEWUNMI *et al.* 1990).

1.3. CONCLUSION

Despite the large number of new plant molluscicides that have been documented, very few actually satisfy the criteria for effective large-scale application (MOTT 1987). Many simply do not have sufficient activity and cannot compete with synthetic molluscicide. Extracts of plants for application to infected sites should originate from regenerating parts, such as fruits and leaves, in order not to destroy the whole plant. Therefore extracts of roots are impractical for mollusciciding.

Of the natural products with the most potential in the fight against schistosomiasis, the triterpene glycosides appear to be in the forefront at the moment, especially as some plant parts can contain as much as 30% saponin.

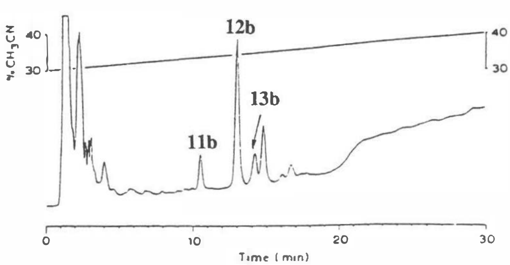
Beside the intensively studied *Phytolacca dodecandra*, two other saponin-containing plants, *Swartzia madagascariensis* and *Tetrapleura tetraptera* could also find practical application. Their wide range of distribution, the large amount of inedible, molluscicidal fruits produced and the toxicity to snails exhibited by their constituents make them viable plant molluscicides. These plants should not provide a universal solution for the control of schistosomiasis, but in an integrated program, involving improvements in standards of hygiene, together with chemotherapy they should help to reduce the transmission of the parasitic disease in endemic areas.

2. Analytical aspects of plant molluscicides

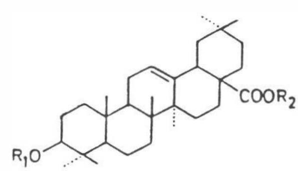
As shown in the first part of this paper, some of the most promising molluscicidal plants for local control of schistosomiasis are saponin-containing plants (*P. dodecandra*, *S. madagascariensis*, *T. tetraptera*, *S. sesban*, etc.). However, care must be taken to investigate the mammalian toxicity and the impact on the environment of these natural products before successful snail eradication schemes can be considered.

A high-performance liquid chromatographic (HPLC) method has been developed for the analysis of saponins in *P. dodecandra* (Domon *et al.* 1984), but it was also necessary to have a rapid method to determine quantitatively the content of saponins of such molluscicidal plants.

Among numerous reasons for an efficient analytical method, the need to investigate plant material from different strains and geographical locations was primordial. At the same time, quantitative analysis of different extraction methods, involving changes in solvent system, temperature and time was very important, in order to maximize the yield of molluscicidal saponins for the most effective treatment of infected sites. Finally, information on the content of



Lichrosorb RP-8 (250 x 4.6 mm); 30-40 % MeCN over 40 min; 1.5 ml/min; 206 nm.



	R_1	R_2
11b	Rha-Glc-Glc- Glc	Glc-
12b	Glc-Glc- Glc	Glc-
13b	Gal-Glc- Glc	Glc-

Fig. 2. — HPLC analysis of aqueous extract of *Phytolacca dodecandra* berries.

active saponins in treated water is essential for biodegradation and toxicological studies.

Separations on reversed-phase columns have previously been carried out with detection at 206 nm owing to the poor absorption of saponins at higher wavelengths (DOMON *et al.* 1984). Consequently, there are limitations concerning the solvents and gradients that can be used. For example, HPLC of an aqueous *P. dodecandra* extract with an acetonitrile-water gradient demonstrates the problem of baseline drift at 206 nm (Fig. 2).

The bidesmosidic saponins (**11b**, **12b** and **13b**) elute between 10 and 20 min but the monodesmosidic saponins (**11**, **12**, and **13**; $R_2 = H$ in Fig. 2) elute later and are consequently much more difficult to quantify. When a gradient solvent system is used, refractive index detection is not practicable, thus an alternative is to derivatize the saponins with a chromophore which facilitates UV detection at 254 nm. As the monodesmosidic saponins from *P. dodecandra*, as well as those from *S. madagascariensis* or *T. tetrapleura* (which are responsible for the molluscicidal activity) possess a free carboxyl group at the C-28 position, derivatization can be carried out at this function. Encouraging results have been obtained by derivatization of the saponins with 4-bromophenacyl bromide in presence of a crown ether (Fig. 3). This method has previously been employed for the analysis of fatty acids and prostaglandins. Details of the procedure for saponins have been published (SLACANIN *et al.* 1988a).

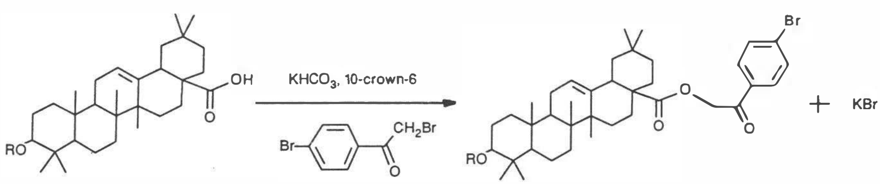


Fig. 3. — Example of derivatization of a saponin with 4-bromophenacyl bromide.

Analysis of a derivatized extract of *P. dodecandra* was achieved by HPLC on a reversed-phase octadecylsilyl column with detection at 254 nm, using a gradient of acetonitrile in water, without any baseline drift (Fig. 4).

For quantification of the saponins, two methods could be considered: (a) use of derivatives of previously isolated saponins as

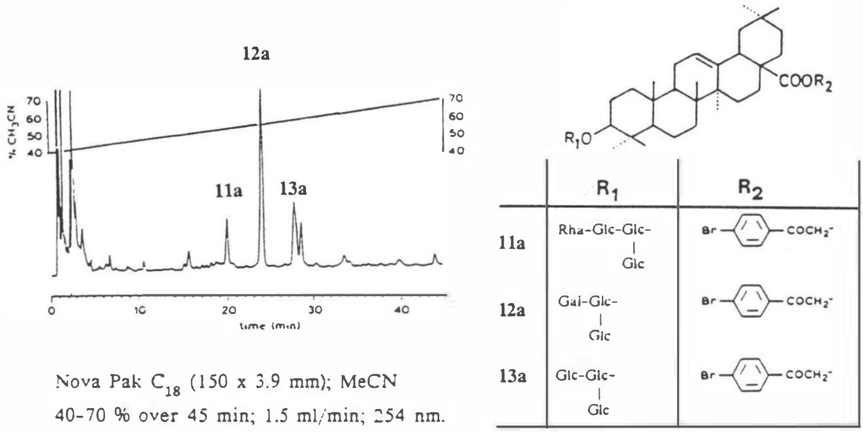


Fig. 4. — HPLC analysis of a derivatized aqueous extract of *P. dodecandra* berries.

external standards; (b) use of naphthalene as internal standard. Comparison of the results showed virtually identical values for the two methods. Consequently, method (a) was employed throughout. Mixtures of selected derivatized saponins (**11a**, **12a** and **13a**) were injected at different concentrations into the HPLC column, and the surface area under each peak was plotted against concentration in order to obtain calibration curves. Calculation of the percentages of saponins in any extract is therefore a relatively straightforward matter, after derivatizing the extract and performing a preliminary purification step (simple filtration over RP-18) before HPLC analysis.

The bidesmosidic saponins lack a free carboxyl functionality and therefore cannot be derivatized by the method employed for the monodesmosidic saponins. Although this class of saponins was inactive in the molluscicidal assay (HOSTETTMANN *et al.* 1982), it is important to quantify these glycosides since they may act as prodrugs in the environment in question. For example, enzymes are present in the crude plant extracts and these are able to hydrolyse the ester linkage in the presence of water, yielding the active monodesmosidic derivatives (DOMON & HOSTETTMANN 1984). Furthermore, hydrolysis may occur in the treated water source. HPLC analyses were performed at 206 nm, without any chemical derivatization. By constructing calibration graphs with selected pure bidesmosidic glycosides, the percentages of each of them in the extracts could be ascertained.

These two methods (with and without derivatization of the extracts with 4-bromophenacyl bromide) were used to quantify saponins in the molluscicidal plant *P. dodecandra.* A comparison of different aqueous extraction procedures for the berries of this plant showed that measurable amounts of monodesmosidic saponins were obtained uniquely at ambient temperatures (Table 3). Extractions with hot water produced only bidesmosidic saponins, presumably owing to inactivation of the enzymes responsible for cleaving the glycosidic chain in position C-28 of the triterpene moiety. This important observation is relevant to the problem of schistosomiasis as obviously only cold water extracts (which contain monodesmosidic active saponins) will have any application as plant molluscicides. Thus, pounding the berries with cold water, the most practicable method of obtaining a vegetable molluscicide in endemic regions, conveniently provides the greatest concentration of saponins for application to sites of infestation by transmitter snails (SLACANIN *et al.* 1988a).

Table 3

Percentages of saponins in Phytolacca dodecandra *aqueous extracts*

Method	Total weight of extract (g)	Saponin in total extract (%, w/w)						
		11a	**12a**	**13a**	**11b**	**12b**	**13b**	Remaining saponins
20 °C, 24 h	0.413	3.5	18.9	3.6	*	*	*	5.4
90 °C, 24 h	0.533	—	—	—	6.4	28.8	4.3	10.8
100 °C, 2 h	0.500	—	—	—	8.3	32.9	4.5	9.9
100 °C, 12 h	0.573	—	—	—	6.2	27.2	4.0	9.8

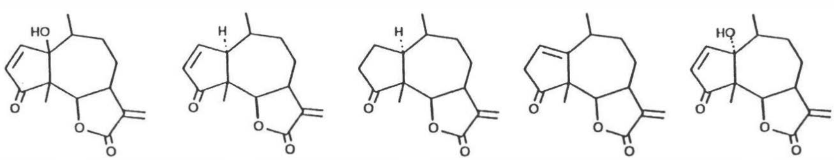
* Bidesmosidic saponins not calculated by this procedure.

For toxicological and other investigations, it is therefore easy to reproduce field extraction conditions of molluscicidal plant parts and then rapidly, by HPLC, obtain standard extracts for their respective active saponins, before submission to required tests.

The HPLC procedure involving derivatization with 4-bromophenacyl bromide can be extended to any saponins, provided either the aglycone or the sugar moiety contains a free carboxyl group. For instance, the saponins from the fruits of *S. madagascariensis* can easily be derivatized by the method described (Borel 1987).

Another plant that enters into the category of promising plant molluscicides is *Ambrosia maritima* (Asteraceae), an annual herbaceous plant widely distributed throughout the Mediterranean region. An important part of the seminar «Vector control of schistosomiasis using native African plants» (Brussels, 24 March 1992) is dedicated to this plant and information about its morphology, its chemical composition, its toxicity and its activity in molluscicidal assays can be found elsewhere in this book. Before the analysis of this plant is described, one or two generalities will be outlined.

Several compounds, in particular the sesquiterpene lactones ambrosin (**15**) and damsin (**16**), have been isolated from the crude herb *A. maritima* (El Sawy *et al.* 1984) and found to have molluscicidal activity. Further phytochemical investigations have revealed the presence of additional constituents, including the sesquiterpene lactones neaombrosin (**17**), hymenin (**14**) and parthenin (**18**) (Abdel Salam *et al.* 1984, Picman *et al.* 1986).



14 **15** **16** **17** **18**

Analytical high performance liquid chromatography (HPLC) of sesquiterpene lactones has not been extensively investigated, but Slacanin *et al.* (1988b) reported an HPLC method for the determination of sesquiterpene lactones in *A. maritima.* As it is the case for saponins from *P. dodecandra* or *S. madagascariensis*, the quantification of the molluscicidal principles of *A. maritima* is necessary for the following reasons:

— The content of molluscicidal sesquiterpenes in plant material from different strains and geographical locations needs to be known;
— It is essential to investigate the efficiency of different extraction modes in order to introduce an optimized procedure; this requires

varying certain parameters, such as solvent, extraction time and temperature;

— Caution should be exercised in the use of sesquiterpene-containing plants, especially those with sesquiterpene lactones, as they are known to be irritating and allergenic to humans; thus there is a pressing need for toxicological studies;

— Determination of amounts of sesquiterpene lactones is required for biodegradation studies and for preparation of standardized extracts.

The major sesquiterpene lactones (**14** - **17**) from *A. maritima* had first to be isolated by several chromatographical techniques, in order to have the pure standards for qualitative and quantitative determinations.

HPLC analyses were carried out on a reversed-phase column, using an acetonitrile-water gradient as mobile phase. Naphthalene was used as internal standard because its UV maximum at 220 nm corresponded well with UV maxima recorded for the sesquiterpenes. Another advantage was that naphthalene eluted after the sesquiterpene lactones and did not overlap with any peaks (Fig. 5).

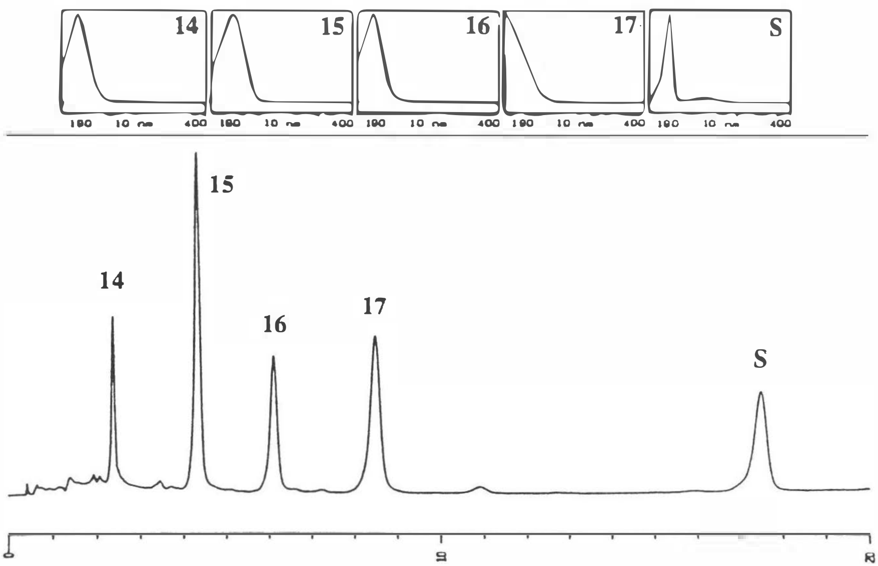
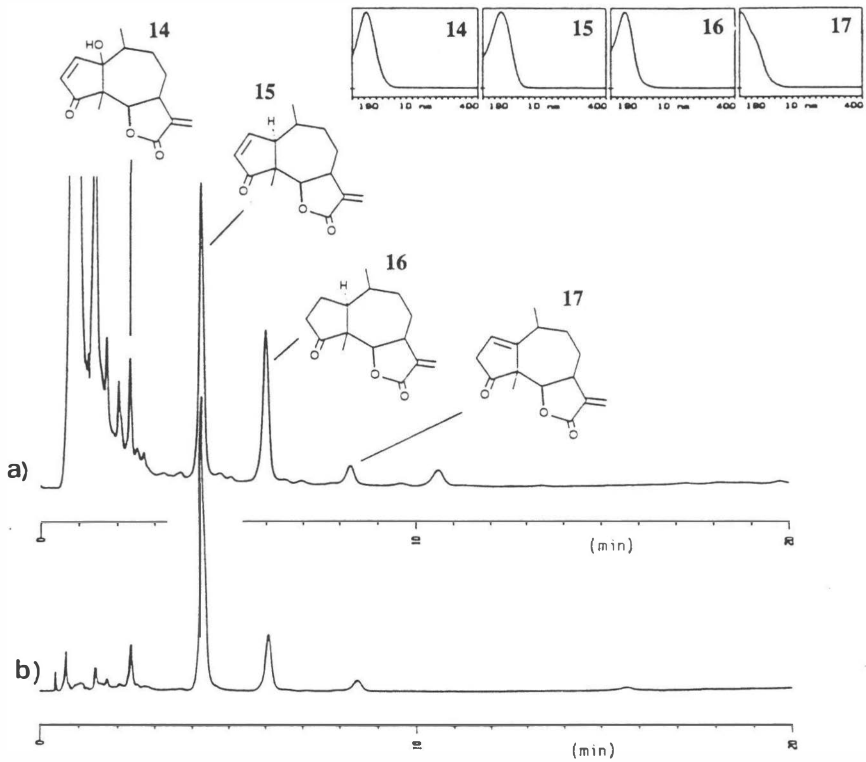


Fig. 5. — Separation of the pure sesquiterpene lactones **14** - **17** (Conditions as in Fig. 6).



Column: RP-8 5 μm (125 x 3.9 mm); Mobile phase: CH_3CN-H_2O 35:65 over 10 min, then 35:65 → 40:60 from 10 to 20 min; Flow-rate: 1 ml/min; Detection: 254 nm

Fig. 6. — a) HPLC-UV analysis of an aqueous extract of *A. maritima.*
b) HPLC-UV analysis of a chloroform extract of *A. maritima.*

Comparison of the HPLC traces of a water extract of *A. maritima* (stirring 12 h at room temperature) and a chloroform extract (stirring 24 h at room temperature) of this plant showed the same most abundant sesquiterpene lactones ambrosin (**15**), damsin (**16**) and hymenin (**14**), while neoambrosin (**17**) occurred, in both extracts, in only a very small amount (Fig. 6).

The weights of extract obtained after extraction with water and chloroform for different time intervals are given in Table 4. It is obvious that much more material was extracted with water than with chloroform. What was more surprising was that after a maximum of 12 h, the amount of aqueous extract subsequently decreased after

24 h, 48 h and 7 days. Table 4 also gives the results obtained from the determination of sesquiterpene lactones **14** - **17** after extraction for different time intervals. These values are expressed as percentages of the total extracts. After 7 days, none of the four sesquiterpene lactones was detectable by the HPLC method. Instead products of degradation were observed in the HPLC trace.

Table 4

Sesquiterpene lactone content of Ambrosia maritima *extracts*

Extract	Extraction time (h)	Total weight of extract (g)	Sesquiterpene lactones (%, w/w)			
			14	**15**	**16**	**17**
Aqueous	6	0.3066	1.15	1.18	1.13	0.2
	12	0.3185	1.28	1.38	1.29	0.22
	24	0.2730	1.54	1.04	1.45	0.22
	48	0.2592	1.76	0.76	1.52	0.21
	7 days	0.2204	0	0	0	0
Chloroform	24	0.0503	7.98	21.49	9.8	2.17

While the aqueous extract obtained after 12 h was active at 400 mgL^{-1} against snails of the species *Biomphalaria glabrata*, those obtained after 24 h, 48 h and 7 days remained inactive at this concentration. On the other hand, the chloroform extract was active at 200 mgL^{-1}. This was presumably the result of the higher relative content of sesquiterpene lactones in this extract (21.5 % ambrosin, for example).

There was obviously a slow degradation of the sesquiterpene lactones in the aqueous extracts, so that after 7 days none of the four lactones remained. A possible explanation was the weak alkalinity of the aqueous extract, which might cause the degradation. In fact, a solution of ambrosin in water at pH 9 is completely degraded after 48 h.

The information obtained from these studies should help in devising protocols for standardizing extracts of *A. maritima*. This aspect is especially important during the stage of field trials, in order to ascertain the amount of sesquiterpene lactones present in different batches of material, from different countries, from different subspecies and from collections at different periods. Toxicological investigations also require an accurate indication of sesquiterpene lactone content.

In conclusion, we may say that, for any plant which has proved itself to be a useful candidate molluscicide, a reliable analytical method is required to measure the content of active principle at a given stage in the exploitation of the vegetable material. This is especially important for toxicological studies, when the exact composition of the molluscicide needs to be known. In the case of sesquiterpene lactones from *Ambrosia maritima*, the analytical aspects are straightforward but other classes of substance are more difficult to quantify. This is the situation for saponins, which lack a strong chromophore and where detection is not straightforward. Provided they possess a free carboxyl group, pre-column derivatization can be employed to help visualize the constituents in question.

The analytical method can then be used in the selection of plant material, in the preparation of standard extracts and in the evaluation of biodegradation following the treatment of an infested site.

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AMBROSIA MARITIMA: MORPHOLOGY, DISTRIBUTION, GENETIC AND CHEMICAL DIVERSITY

BY

L. TRIEST, M. VAN DE VIJVER, M. EL ARIFI & J.J. SYMOENS *

SUMMARY. — *Ambrosia maritima* L. (Asteraceae) is a plant widely distributed along the coastal zone of the Mediterranean and throughout Asia Minor, Africa and Madagascar. Slight variations in the pubescence of the leaves, the size and shape of fruits and the shape of the fruit spines suggest the existence of several population groups on the African continent, but there are no qualitative diagnostic features allowing to distinguish taxa of subspecific or varietal rank. At the population level, the genetic diversity based on seed isozymes is rather low, whilst current research on leaf isozymes indicates a somewhat larger variability. Within a population, the seeds are mostly very uniform in their enzymes, although allelic variation was observed for leucine aminopeptidase in seeds from Retba; leaf enzymes show somewhat more polymorphism, especially in South Africa, a striking example being the diagnostic patterns for several enzymes in plants from Richard's Bay. Up to now, 21 sesquiterpene lactones were identified in *A. maritima*, ambrosin and damsin being mostly the major compounds. At population level, the chromatograms of plants from Spain, Egypt, Senegal and Ivory Coast show a large uniformity, but in some South African populations, ambrosin and damsin are totally lacking or only present in trace amounts. Within a population, there are no qualitative differences, but quantitative differences occur, depending on the developmental stage. The rather general uniformity in sesquiterpene lactones observed until now may enhance the cultivation of *A. maritima* and justify its use as a molluscicide in different regions.

RÉSUMÉ. — Ambrosia maritima: *Morphologie, distribution, diversité génétique et chimique.* — *Ambrosia maritima* L. (Asteraceae) est une plante largement distribuée dans les régions côtières de la Méditerranée et en Asie Mineure, en Afrique et à Madagascar. De petites variations dans la pubescence des feuilles, les dimensions et la forme des épines des fruits suggèrent l'existence de plusieurs groupes de populations

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sur le continent africain, mais l'absence de caractères qualitatifs diagnostiques empêche d'y reconnaître des taxons du rang de la sous-espèce ou de la variété. Au niveau des populations, la diversité génétique que révèlent les isozymes des graines est relativement faible, tandis que les recherches en cours sur les isozymes des feuilles indiquent une variabilité un peu plus grande. Au sein d'une même population, les graines sont généralement très semblables en ce qui concerne leurs enzymes, bien qu'une variation allélique ait été observée pour la leucine-aminopeptidase dans des graines provenant de Retba; les enzymes des feuilles montrent un polymorphisme un peu plus accentué, spécialement en Afrique du Sud, un exemple frappant étant celui des zymogrammes caractéristiques concernant plusieurs enzymes dans des plantes de Richard's Bay. À ce jour, 21 sesquiterpènes-lactones ont été identifiées chez *A. maritima*, l'ambrosine et la damsine étant généralement les composés principaux. Au niveau des populations, les chromatogrammes des plantes d'Espagne, d'Égypte, du Sénégal et de la Côte d'Ivoire montrent une grande uniformité, mais dans certaines populations d'Afrique du Sud, l'ambrosine et la damsine peuvent être totalement absentes ou seulement présentes à l'état de traces. Au sein d'une même population, il n'y a pas de différences qualitatives, mais bien des différences quantitatives liées au stade de développement. L'uniformité générale assez nette des sesquiterpènes-lactones observée jusqu'à présent devrait favoriser la culture d'*A. maritima* et justifier son emploi comme molluscicide dans différentes régions.

SAMENVATTING. — Ambrosia maritima: *Morfologie, verspreiding, genetische en chemische diversiteit.* — *Ambrosia maritima* L. (Asteraceae) is een wijd verspreide plant in de kuststreken van de Middellandse Zee en in Klein-Azië, Afrika en Madagascar. Kleine variaties in de pubescentie van de bladeren, de grootte en de vorm van de fruitdoornen suggereren het bestaan van verschillende populatiegroepen op het Afrikaanse continent, maar er zijn geen kwalitatieve diagnostische kenmerken die het mogelijk maken er taxa van de rang van de ondersoort of de variëteit te onderscheiden. Op het populatieniveau is de genetische diversiteit onthuld door de isozymen van de zaden betrekkelijk gering, terwijl aan de gang zijnde onderzoekingen over de isozymen van de bladeren een iets grotere variabiliteit vertonen. In éénzelfde populatie zijn de zaden meestal zeer gelijkend in hun enzymen, alhoewel een allelische variatie waargenomen werd voor de leucine-aminopeptidase in de zaden afkomstig van Retba; de enzymen van de bladeren vertonen iets meer polymorfisme, in het bijzonder in Zuid-Afrika. Een opvallend voorbeeld daarvan zijn de diagnostische patronen aangaande verschillende enzymen in planten van Richard's Bay. Tot nu toe werden 21 sesquiterpene-lactonen geïdentificeerd bij *A. maritima*, waarin ambrosine en damsine meestal de voornaamste bestanddelen vormen. Op het populatieniveau vertonen de chromatogrammen van de planten uit Spanje, Egypte, Senegal en de Ivoorkust een grote eenvormigheid, maar in sommige populaties van Zuid-Afrika kunnen ambrosine en damsine volledig afwezig zijn of slechts aanwezig in sporehoeveelheden. In éénzelfde populatie zijn er geen kwalitatieve, maar wel kwantitatieve verschillen afhankelijk van het ontwikkelingsstadium. De tamelijk algemene eenvormigheid in sesquiterpenelactonen die tot nu toe waargenomen werd zou de kweek van *A. maritima* moeten bevorderen en zijn gebruik als molluscicide in verschillende streken moeten rechtvaardigen.

Introduction

Snail control is a somewhat neglected aspect of the control of schistosomiasis. It is, however, an essential element of an integrated approach to prevent this parasitic infection, which remains one of the most important tropical diseases. Few plants have been studied in detail, not only concerning their effect on snails, but also regarding their toxicity to non-target organisms, their phytochemical and phytotechnical aspects, etc. *Ambrosia maritima* L. (Asteraceae) is considered as one of the most promising molluscicidal plants (McCullough & Mott 1983) together with *Phytolacca dodecandra* L'Hérit. (Phytolaccaceae) and *Jatropha curcas* L. (Euphorbiaceae).

We examined the polymorphism of several *Ambrosia maritima* populations from Africa and the Mediterranean area because no study on that scale was available. Before using a plant as a genetic resource it is important to know the taxonomic delimitation of the species, the geographical distribution and the trends towards diversification. The latter study has been initiated using morphological features as well as enzymes and the active principles (sesquiterpene lactones).

Plant material

For the morphological comparison of the plants from different regions, herbarium specimens from the following institutions were studied: BM, BR, BRVU, K, P, PRE, NU and WAG.

For the electrophoretic experiments viable seeds or leafy parts from plants grown from seeds (1988-1990) were used, while bulk air dried leaves were extracted for further chromatographic analyses (Table 1).

Morphology, taxonomy and geographical distribution

Members of the genus *Ambrosia* L. can be distinguished easily from the other members of the Asteraceae because it is the only genus where the male flowers are arranged in numerous capitula on top of the branches, whereas the female flowers are solitary or in clusters in the leaf axils. The genus is species-rich in N. America (Payne 1976), but in Africa and the Mediterranean area, only the aromatic *Ambro-*

Table 1

List of Ambrosia maritima *collections that were used for electrophoretic and chromatographic analyses (a+means that the material was investigated)*

Country	Locality	Seeds used for isozyme analysis	Leaves used for isozyme analysis	Dried leaves used for chemical analysis
Spain	Cadiz	–	–	+
Egypt	Luxor	+	–	+
	Nagada	+	+	+
	Rezegat	+	+	+
	Asment	+	–	+
Senegal	Retba	+	–	+
	Tanma	–	–	+
Ivory Coast	Korhogo	+	+	+
South Africa	St. Lucia	+	–	+
(Natal)	Richard's Bay	+	+	+
	Nseleni	–	+	+
	Port Durnford	–	–	+
	Isipingo (Durban)	+	–	+
	Amanzimtoti	+	–	+
	Winklespruit	+	–	+
	Umkomaas	+	–	+
	Ifafa	+	–	+
	Port Shepstone	+	–	+
	Munster	–	–	+
	Port Edward	+	–	+
	Ixopo	–	–	+

sia maritima is reported as being native. The possible confusion with introduced agricultural weeds such as *Ambrosia artemisiifolia* L. and *Ambrosia trifida* L. has been discussed earlier (TRIEST *et al.* 1989). *Ambrosia maritima* is annual or perennial (FAHMY & DARWISH 1949) and widely distributed along the coastal zone of the Mediterranean and throughout Africa, Madagascar and Asia Minor (Fig. 1). A taxonomic revision based on herbarium material revealed that *Ambrosia villosissima* Forsk. from Egypt and *Ambrosia senegalensis* DC. from Senegal should be regarded as synonyms of *Ambrosia maritima* (VAN DE VIJVER 1987).

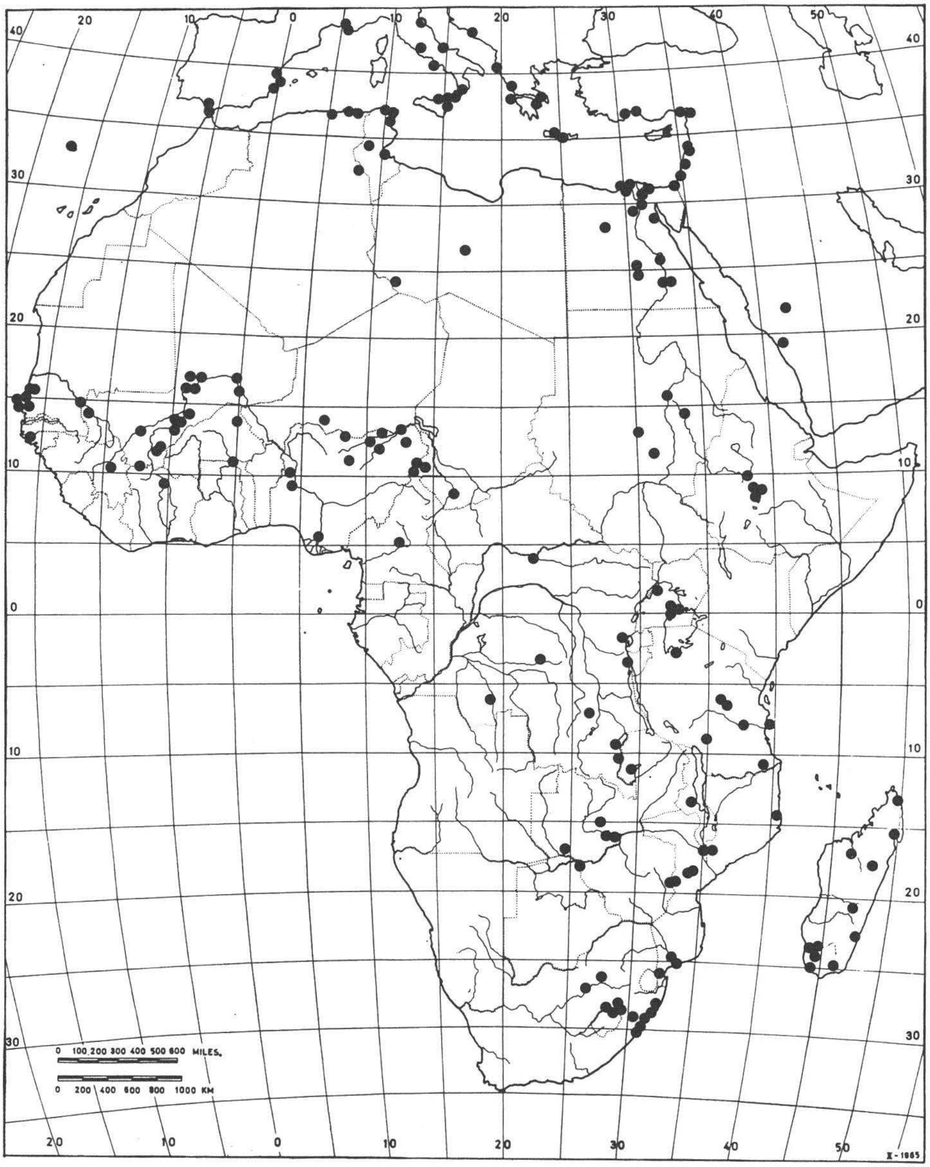

Fig. 1. — The distribution of *Ambrosia maritima*.

Within *Ambrosia maritima* however, the slight diversity of morphological features provides some evidence for the existence of several population groups on the African continent, but there are no qualitative diagnostic features to distinguish subspecies or varieties. This was shown by comparing the number of hairs on the leaves, the size and shape of the blades, the number and density of male capitula, the size and shape of the fruits and the shape of the spines on the fruits (VAN DE VIJVER 1987, TRIEST *et al.* 1989). In general, plants from Asia Minor, northern Africa, the Sudanese region (from Senegal to Ethiopia) have more pubescent leaves than those from eastern, central and southern African populations. An amount of (70 –) 100-110 (– 140) trichomes per mm^2 were counted on plants from the former region, while about (50 –) 70-80 (– 100) trichomes per mm^2 on those from the latter regions (VAN DE VIJVER 1987). Furthermore, plants from the Nile basin have long and unequally distributed spines on the fruits, whereas in the other regions, they are short and distributed more or less on the upper part of the fruit only (TRIEST *et al.* 1989). Nevertheless, despite these minor variations, *Ambrosia maritima* should be regarded as a very uniform species in its morphology, especially when considering such a large distribution area.

Genetical polymorphism

As the overall degree of polymorphism in the morphology of the plants is rather low, we checked the genetic diversity of certain populations by means of enzyme electrophoresis. This technique should give us more information about additional detectable variation in *Ambrosia maritima* when looking at genes instead of exomorphological features. Not only the amount of genetic variation could be detected, but also its partitioning among the individuals of populations from different regions. Especially the use of enzymes as biomarkers of biological features of the plants (eventually the presence or absence of certain sesquiterpene lactones) should allow us to set up sampling priorities for collecting germplasm.

Genetic diversity at population level

The genetic diversity based on seed isozymes is rather low. A genetic distance of 5 % based on 13 loci has been observed (TRIEST *et al.* 1989). Allelic variants within a population were observed for ADH (alcohol dehydrogenase) in Egypt and for LAP (leucine aminopeptidase) in Senegal, but in general, seed isozymes are very uniform among these populations from remote areas. Continued investigations with 15 enzymes, representing 21 putative loci, failed to indicate additional allelic variants in seeds. Current research on leaf isozymes indicates a somewhat larger variability. Up to now, a number of enzymes show different electromorphs at the level of the collections from Egypt, Ivory Coast and South Africa. For example, SkDH (shikimate dehydrogenase), POD (peroxidase) and EST (esterase) have different isozymes in plants from Egypt and Ivory Coast, while ME (malic enzyme), GOT (glutamate-oxaloacetate-transaminase) and EST are diagnostic for the South African populations. These genotypes show not only divergences on a macrogeographic scale but are most polymorphic among the South African populations. At this moment, no correlation between the enzyme polymorphism and either leaf or fruit morphology has been observed. This could perhaps be a result of the low sample size on African scale.

Genetic diversity at the individual level

The genetic diversity (even if it is low) based on seed enzymes in *Ambrosia maritima* is mainly distributed between populations and not among the individuals within a population. Nevertheless, allelic variation was observed for LAP in seeds from Retba (Senegal), indicating cross-pollination under natural conditions in annual plants (TRIEST *et al.* 1989). The fact that no real allelic variation was observed in seeds from populations in Egypt and South Africa most probably has to do with the perennial character of the collected specimens. In general, for *Ambrosia maritima*, one may assume that the seeds are very uniform in their enzymes (except LAP and ADH), but that the leaves exhibit somewhat more polymorphism. A striking example of the latter is the fairly higher amount of diagnostic patterns in plants from Richard's Bay (South Africa). These plants clearly belong morphologically to *Ambrosia maritima*, but show deviating patterns when compared to the other South African populations. The Richard's Bay population showed diagnostic patterns for POD, LAP, SkDH and 6PGD (6-phosphogluconate dehydrogenase). Maybe one of these enzymes can be used as a marker for the sesquiterpene lactone content, because this population lacked both

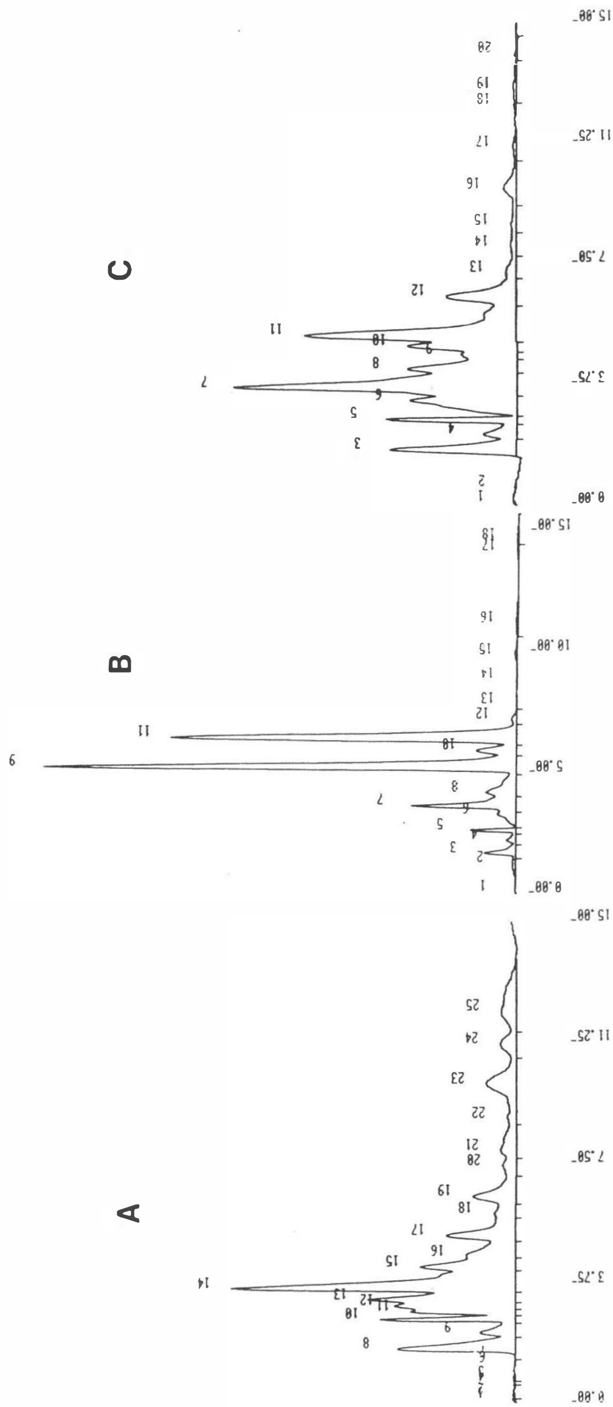

Fig. 2. — The reproducibility of an analytical method to purify the sesquiterpene lactone fraction. A : hexane-ether (2 : 1 ; B : hexane-ether (1 : 3) ; C : ether-methanol (9 : 2).

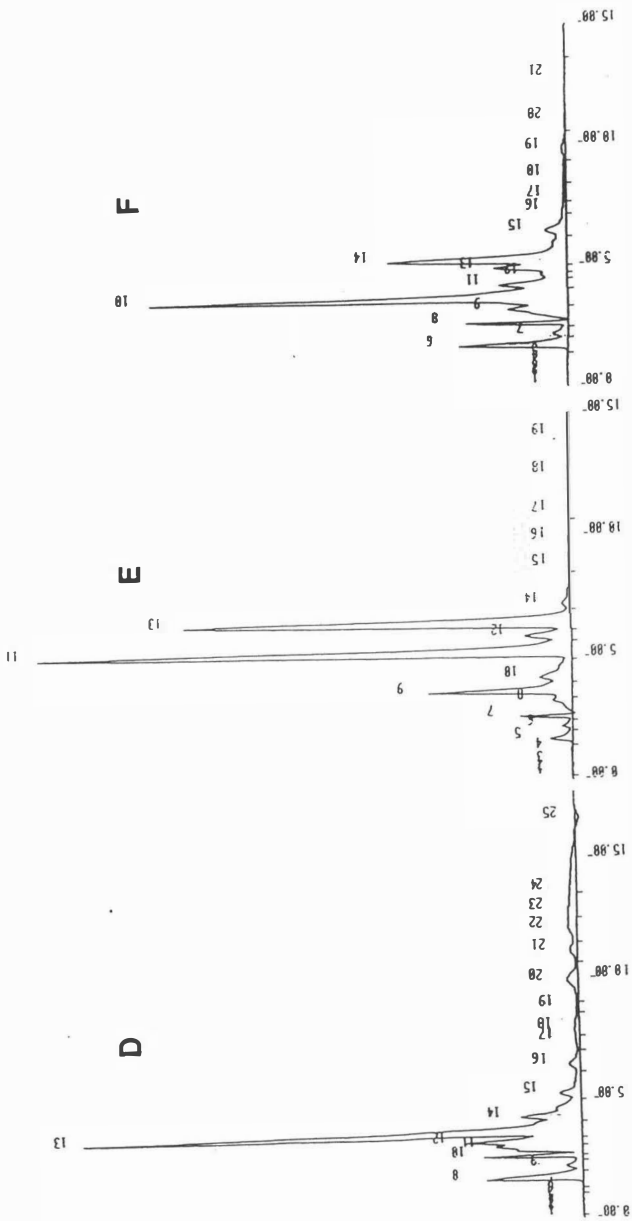

Fig. 3. — The reproducibility of an analytical method to purify the sesquiterpene lactone fraction. D : hexane-ether (2 : 1); E : hexane-ether (1 : 3); F : ether-methanol (9 : 2).

ambrosin and damsin and showed a low overall amount and diversity of sesquiterpene lactones.

Chemical polymorphism

The variation in sesquiterpene lactone content is of special interest, because these substances are the main (if not the only) compounds extracted from *Ambrosia maritima* with molluscicidal activity. Our study deals with this chemical variation on a fairly large geographical scale, using plants from 21 populations originating from Spain, Egypt, Senegal, Ivory Coast and South Africa. We were also interested to know the variation at different growth stages of the plants.

Methods

The preparative isolation of pure sesquiterpene lactones has been modified after JAKUPOVIC *et al.* (1987). A hexane-ether-methanol (1 : 1 : 1) extract of the grinded leaves was flash chromatographed on silica gel, eluting first with hexane, then changing gradually to ether and methanol. The fractions with sesquiterpene lactones were further purified on a series of columns (subsequently ether, hexane-acetone 7:3, chloroform-methanol 20:01) and finally on preparative HPLC using lichrospher 100 RP18 (5 µm) columns with methanol-water (9:2) as solvent at a flow-rate of 1 ml/min. The U.V. detection was at 220 nm. To purify ambrosin and damsin, a two-dimensional TLC method was also developed, using subsequently hexane-acetone (6:4) and chloroform-methanol (20:01).

Reproducibility

A rapid and reproducible method for the analytical screening of sesquiterpene lactones in *Ambrosia maritima* was developed. The grinded leaves (100 mg) are extracted in hexane-ether (1 : 3), placed on adsorbex columns (Si-100 mg), and then further eluted with the following series of solvents: 1. hexane; 2. hexane-ether (2:1); 3. hexane-ether (1:3); 4. ether-methanol (9:2); 5. methanol. The hexane-ether (1:3) fractions contain the sesquiterpene lactones and are further purified on an adsorbex column (RP8-100 mg) before injecting for HPLC analysis. The reproducibility of the technique is shown in Figs. 2 & 3.

Amount of sesquiterpene lactones

Sesquiterpene lactones are essentially present in the leaves and flowering heads, rarely in the stems or roots. Up to now, 21 sesquiterpene lactones have been identified, but ambrosin and damsin are the major compounds (ABU-SHADY & SOINE 1953, SALAM *et al.* 1984, PICMAN *et al.* 1986, JAKUPOVIC *et al.* 1987, SLACANIN *et al.* 1988). Depending on the time of collection and on the heterogeneity of the plant material (only leaves and stems, or a mixture of vegetative structures with flowering heads and fruits), up to 2.2% sesquiterpene lactones of the total DW from Egyptian and Senegalese plants could be purified when using the above mentioned preparative separation method. On an average for a harvest with mixed individuals one may assume that ambrosin and damsin are the major compounds and that each represents between 20-40 % of the total amount of sesquiterpene lactones. Nevertheless there may be qualitative variations depending on the geographic origin of the plants and quantitative variations depending on the time of collecting (vegetative, in flower or in fruit).

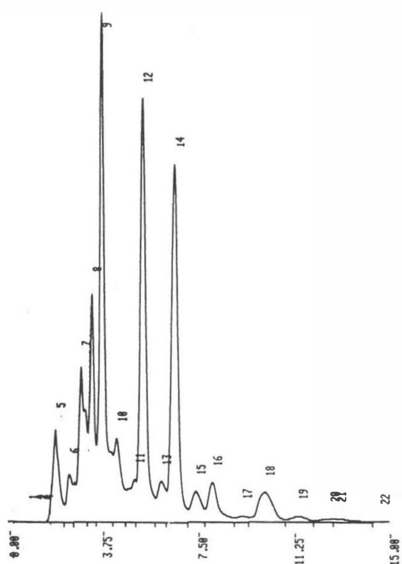

Fig. 4. — HPLC separation of the sequiterpene lactone fraction in *Ambrosia maritima* from Cadiz (Spain).

Variation of sesquiterpene lactones at the level of populations

The chromatograms of *Ambrosia maritima* plants from Spain, Egypt, Senegal and Ivory Coast show a similar set of peaks for the sesquiterpene lactone fraction, indicating a large (if not a total) uniformity (Fig. 4). Ambrosin and damsin are always present in the leafy parts of plants from these areas, but might be absent in certain South African populations (Fig. 5). The figures represent the amount of ambrosin and damsin in the leaf blades (thus not the entire aerial part of the plant) of individuals from seven natural populations and 2 cultures. Both compounds that normally are the major constituents of the sesquiterpene lactone fraction are totally lacking or only present in trace amounts in *Ambrosia maritima* plants collected at Richard's Bay, Nseleni, Port Dunford, Winklespruit and Umkomaas. The major sesquiterpene lactone in these plants still needs identification.

Variation of sesquiterpene lactones at the level of individuals within a population

When comparing the chromatograms of the individuals at a single growing place, there are no qualitative differences between

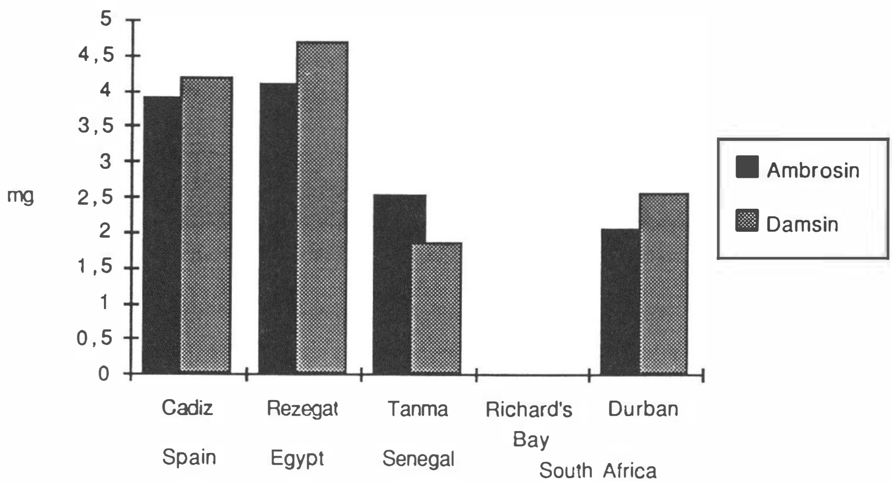

Fig. 5. — The amount of ambrosin and damsin present in 1 g (dry weight) plant material from different origins, compared at the individual level. Both compounds can be absent in South African populations.

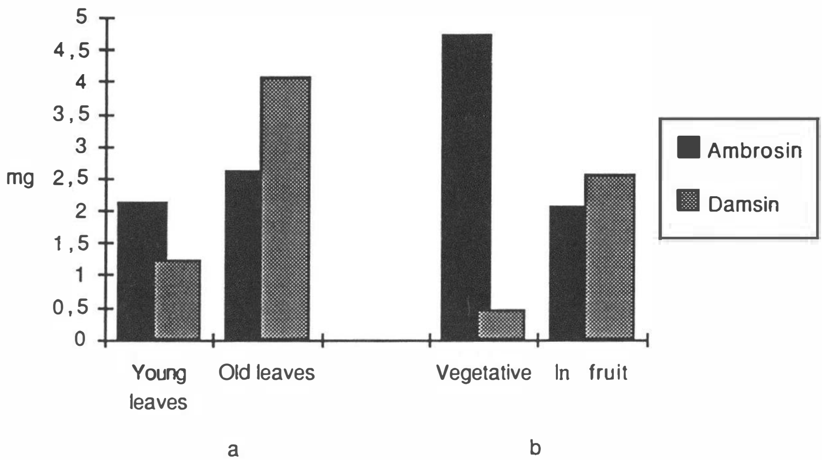

Fig. 6. — The amount of ambrosin and damsin present in 1g (dry weight) plant material: a) compared between young and old leaves of the same individual (an Egyptian strain cultivated at the Centre Universitaire Dschang, Cameroon); b) compared between an individual in the vegetative phase (St. Lucia, South Africa) and an individual in fruit (Durban, South Africa).

juvenile plants and the fully developed plants in vegetative stage, flowering or fruiting. The chromatograms show the presence of similar compounds, but there are large quantitative differences. E.g. the ratio ambrosin: damsin is > 1 in young leaves, but is < 1 in older leaves of the same individual (Fig. 6). Similar results were obtained when comparing entire young plants with those that are in flower or in fruit. One may give here the example of the ambrosin content in 7 individuals from the estuary near St. Lucia (South Africa). About 5 mg ambrosin is present in the leaves and stem of a plant that just started flowering, whereas a much lower amount (0.07-1.4 mg) was observed in the clustered leaves and the stems of plants bearing fruits (Fig. 7).

Conclusion and recommendations

When considering *Ambrosia maritima* as a species, largely distributed along the Mediterranean coast and throughout Africa and Madagascar, it shows a very low degree of polymorphism. This is true for the morphology, as well as for the enzyme polymorphism and for the sesquiterpene lactone variation of the plant. The main

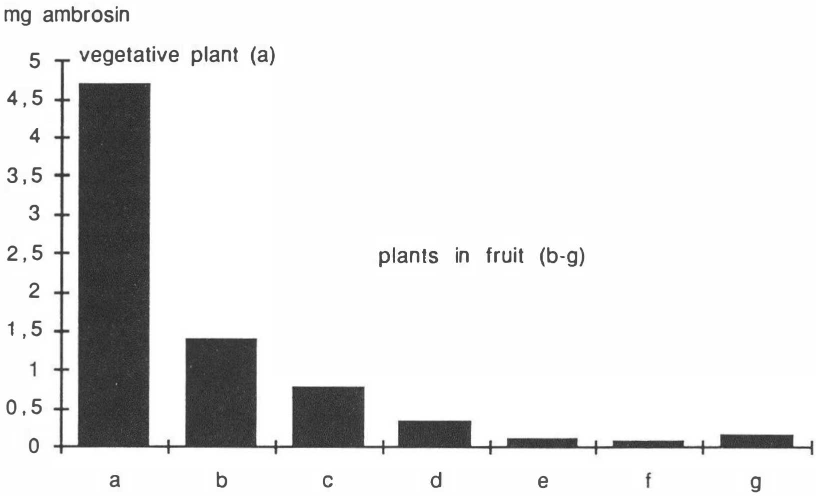

Fig.7. — The amount of ambrosin present in 1 g (dry weight) plant material, compared between individuals from the same population (St. Lucia, South Africa).

differences in the kind and the amount of active principles observed up to now are in Natal (South Africa).

The research is still going on with plant material cultivated under controlled conditions, to find out which part of the considerable variation in the amount of secundary metabolites (and leaf enzymes) is due to genetic variability, origin, time of collection and way of storage. A selection of the individuals with the highest yield of sesquiterpene lactones has to be made.

Additional germplasm from especially NE and SE Africa is needed to verify the extent of the large uniformity in sesquiterpene lactones, observed until now. Such a uniformity certainly may enhance the cultivation and use of *Ambrosia maritima* as a molluscicide in different regions because its 'introduction' into areas where the plant is rare or absent becomes more justified. The species is often locally common, but large distances between populations may exist so that introductions could become a necessity. Nevertheless, not only phytotechnical studies are urgently needed in order to determine the most convenient cultivation technique, the yield and the optimal moment of harvesting, but also ecological studies are required to estimate the possible weedy status of the species.

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Seminar
« Vector control of schistosomiasis using native African plants »
(Brussels, 24 March 1992)
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Royal Academy of Overseas Sciences (Brussels)
pp. 79-87 (1992)

STRUCTURE OF THE SESQUITERPENE LACTONES OF *AMBROSIA MARITIMA*

BY

J.C. BRAEKMAN & A. PEREIRA*

SUMMARY. — If *Ambrosia maritima* enters the category of promising molluscicides, it is due, at least in part, to its ability to synthesize a large assemblage of sesquiterpene lactones. As early as 1953, two of these compounds, damsin and ambrosin were obtained under crystalline form from flowering tops of *A. maritima*. Until now, 18 different sesquiterpene-lactones were isolated from *A. maritima*, 14 of them having been found to have the ambrosanolide skeleton. In this paper, the structures of these compounds are given, and the possible relationships between their biological activities and some of their structural features are discussed.

RÉSUMÉ. — *Structure des lactones sesquiterpéniques* d'Ambrosia maritima. — Si *Ambrosia maritima* entre dans la catégorie des plantes prometteuses en tant que molluscicides, elle le doit au moins en partie à son pouvoir de synthétiser un large assemblage de lactones sesquiterpéniques. Dès 1953, deux de celles-ci, la damsine et l'ambrosine, furent obtenues à l'état cristallin à partir d'inflorescences d'*A. maritima*. A ce jour, 18 lactones sesquiterpéniques différentes ont été isolées d'*A. maritima*, dont 14 ont le noyau ambrosanolide. Dans le présent article, les structures de ces composés sont décrites, et les relations possibles entre les activités biologiques et certaines particularités structurales de ces lactones sont discutées.

SAMENVATTING. — *Structuur van sesquiterpene-lactonen van* Ambrosia maritima. — Als *Ambrosia maritima* toetreedt tot de categorie van de veelbelovende molluscisiden, dankt zij het ten minste voor een deel aan haar vermogen om een wijde samenstelling van sesquiterpene-lactonen te synthetiseren. Reeds in 1953 konden twee van deze samenstellingen, de damsine en de ambrosine, bekomen worden in kristaltoestand vanuit de bloeiwijze van *A. maritima*. Tot nu toe werden 18 verschillende sesquiterpene-lactonen geïsoleerd van *A. maritima*, waarvan 14 de ambrosanolide kern

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bezitten. In dit artikel worden de structuren van deze samenstellingen beschreven, en de mogelijke verbanden tussen de biologische activiteiten en bepaalde structurele kenmerken van deze lactonen worden besproken.

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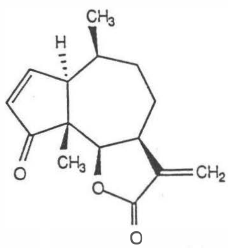
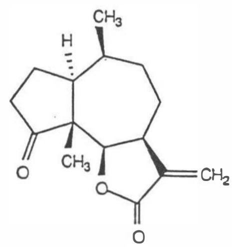
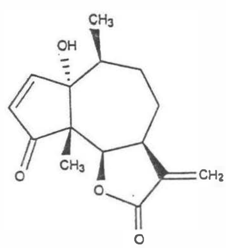
If *Ambrosia maritima* enters in the category of promising plant molluscicides, it is due at least in part to its ability to synthesize a large assemblage of structurally related sesquiterpene lactones. Our



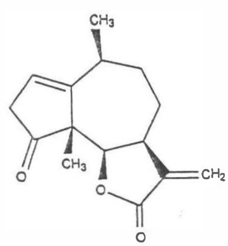
1 Ambrosin



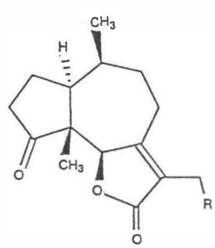
2 Damsin



3 Parthenin

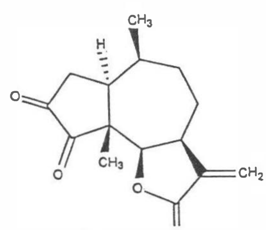


4 Neoambrosin



5 R = H

6 R = OH



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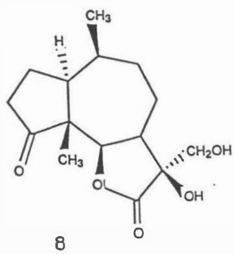

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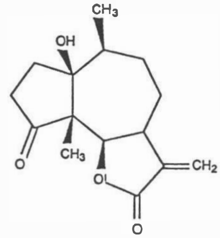
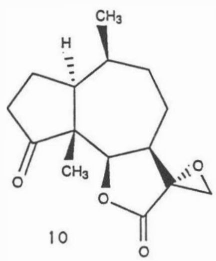


9 HYMENIN



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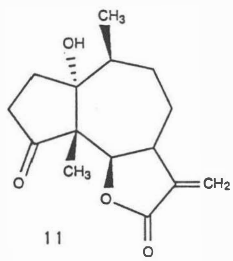

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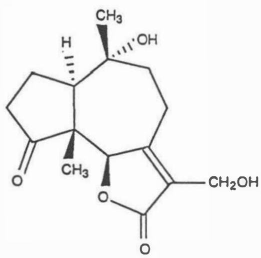

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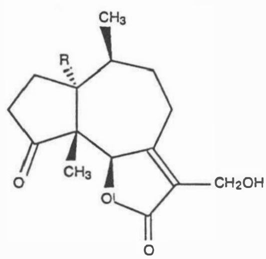
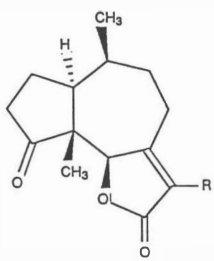
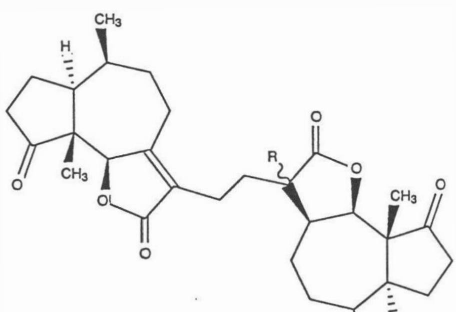


13 R = H
14 R = OH



15 R = H
16 R = OH



17 R = α OH
18 R = β OH

purpose here is to review briefly the available information regarding the structure and the biogenetic origin of these lactones. We will also discuss shortly, the possible relationships between their biological activities and some of their structural features.

The first report on the constituents of *A. maritima* is a paper of ABU-SHADY and SOINE (1953). In this paper the authors describe the isolation, from dried flowering tops of the plant obtained from a druggist in Cairo, of two crystalline compounds named damsin and ambrosin. These compounds are the two main constituents of the ethanolic extract. It was only nine years later that, on the basis of ^{1}H NMR data and chemical correlation with parthenin, the structures of these two sesquiterpene lactones were correctly formulated as **1** and **2** respectively (HERZ *et al.* 1962).

Later on, SALAM *et al.* (1984) reported, in addition to damsin and ambrosin, the isolation from air-dried aerial parts of *A. maritima* collected near Alexandria (Egypt) of six further related sesquiterpene lactones: parthenin (**3**) and neoambrosin (**4**) which had been already isolated from other plants and the new lactones **5** to **8**. A few times later PICMAN *et al.* (1986) found in a sample of *A. maritima* collected in the lower Nile Delta: ambrosin, damsin and hymenin (**9**) the epimer at C-1 of parthenin.

More recently, the investigation by JAKUPOVIC *et al.* (1987) of two collections of *A. maritima*, one from Senegal and the other from Egypt, afforded in addition to seven of the lactones reported previously, nine new ones (**10-18**) including two nor-sesquiterpenes (**15**, **16**) and two dimeric lactones (**17**, **18**). The two collections gave the same set of lactones but in different concentrations.

Thus, until now, 18 different sesquiterpene lactones have been isolated from *A. maritima*, 14 of them having been found to have the ambrosanolide skeleton. They differ from each other, only by their degree of oxydation. This is compatible with the observation that an individual plant species generally yields only one skeletal type, with oxidative variations on that skeleton. In addition to this homogeneous group of compounds four structurally closely related derivatives have also been isolated: compounds **15** and **16** which are nor-ambrosanolides arising from the loss of the methyl group located on the γ-lactone ring, and compounds **17** and **18** which are dimers of the fundamental skeleton.

That *A. maritima* elaborates sesquiterpene lactones is not surprising since these natural compounds are considered to be characteristic constituents of Compositae. They occur in most tribes and genera of this very large family of flowering plants (FISCHER *et al.* 1979). Moreover, the sesquiterpene lactones pertaining to the ambrosanolide group usually occur in the subtribe Ambrosiinae and in the genus *Parthenium.*

Biogenetically, the ambrosanolide skeleton can be considered as deriving from the germacrane skeleton as shown in Fig. 1, the latter

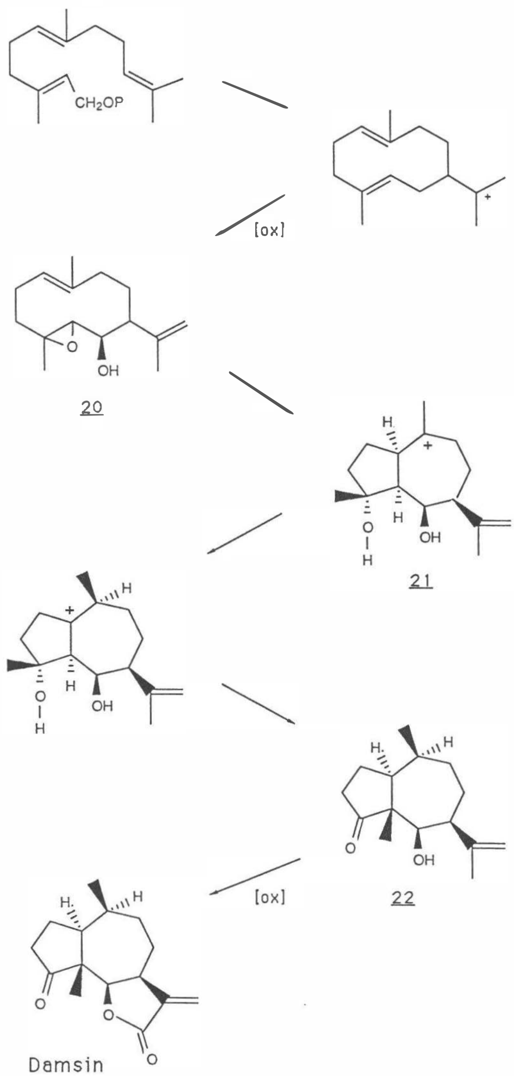


Fig. 1. — Proposed biogenetic scheme of damsin.

skeleton being itself generated by straightforward cyclization of trans, trans-farnesyl pyrophosphate, the precursor common to all sesquiterpenes. The intermediate epoxide **20** will initially undergo a Markornikov cyclization to the cation **21**, which upon double hydride and methyl shift gives the intermediate **22** from which damsin may be easily formed. Damsin can be regarded as the parent lactone from which all the other *Ambrosia maritima* lactones can be generated upon further oxydation.

As already mentioned, the structure of the *A. maritima* sesquiterpenes are characterized by a γ-lactone ring closed toward C-6 and a ketone function at C-4. In several compounds these carbonyl groups are α,β-unsaturated. Such systems are very sensitive to nucleophilic agents, giving rise to 1,4-addition reaction (Fig. 2). In particular,

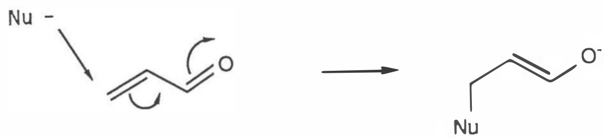


Fig. 2. — 1,4-Addition of a nucleophile to a α,β-unsaturated carbonyl group.

their reactivity toward thiols and amines is well documented. Thus, helenalin which contains both α-methylene-γ-lactone and cyclopentenone moieties, forms bis adduct with reduced glutathione as depicted in Fig. 3 (IVIE & WITZEL 1983). These facts may explain the

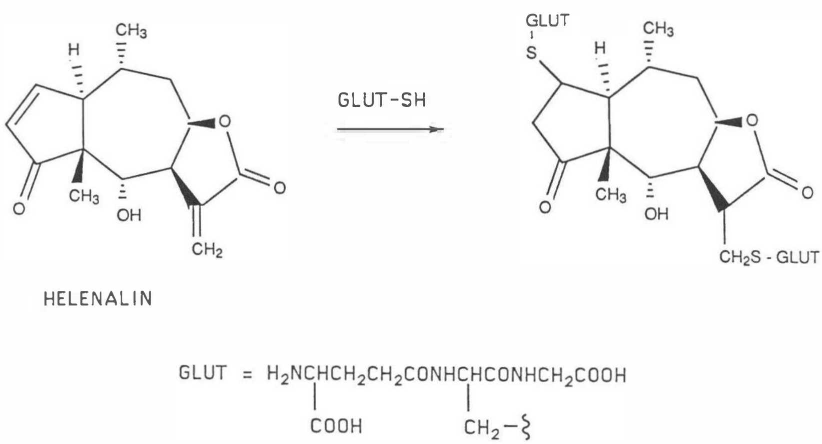


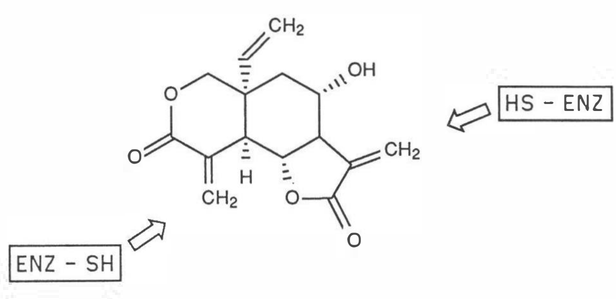
Fig. 3. — Reaction of helenalin with glutathione.

observation made by SLACANIN *et al.* (1988) that the amount of aqueous extract of *A. maritima* diminishes with increasing time of extraction. After seven days none of the initial sesquiterpene lactones are detectable in the extract. Instead, a degradation product of still unknown structure is obtained as major product. The same authors also point out that a solution of ambrosin in water at pH 9 is completely degraded after 48 h.

On the basis of studies to date, it is apparent that sesquiterpene lactones are highly biologically active compounds that may affect a wide variety of microbial, plant and animal systems (IVIE & WITZEL 1983; RODRIGUEZ *et al.* 1976). In many cases it has been demonstrated that the biological activity is usually, but not always, associated with the presence of an α-methylene-γ-lactone. When an additional unsaturated lactone is present, higher degrees of biological activity are observed. It has been repeatedly proposed that these activities are closely related to the efficient way these lactones can alkylate thiols and amino groups in biological systems and particularly in proteins containing cystein and lysine residues.

The following selected examples taken from the review of IVIE & WITZEL (1983) devoted to the biological action of sesquiterpene lactones, illustrate this statement.

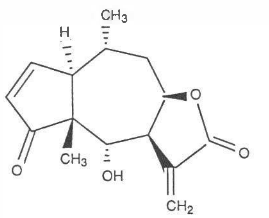
1. Vernolepine (**23**) which is a potent antitumor agent, inhibits the sulfhydryl enzyme phosphofructokinase, apparently by reaction with the thiol groups of the enzyme (Fig. 4).



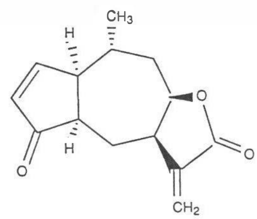
VERNOLEPIN

Fig. 4. — Inactivation of vernolepin by enzyme SH groups.

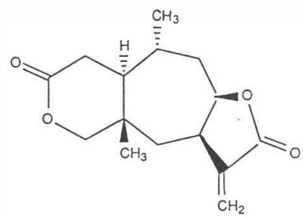
2. Among a large number of sesquiterpene lactones tested for allergenic potential in human skin patch tests, the α-methylene-γ-lactone moiety was found to be the principal group responsible for allergenic effects. Reduction of the conjugated double bond renders the compound inactive.
3. High acute mammalian toxicity of plants containing sesquiterpene lactones is often associated with the presence of compounds that possess two reactive functional groups. Thus, the toxicity of helenalin and mexicanin-E is about ten times higher than that of psilotropin (Fig. 5).



HELENALIN (10)



MEXICANIN-E (3)



PSILOTROPIN (112)

Fig. 5. — Comparison of the LD_{50} of some sesquiterpene lactones (mg/kg mouse): helenalin and mexicanin-E with two reactive functional groups are about ten times more toxic for mammals than psilotropin.

Despite these examples, it has to be mentioned that in some cases, the biological activity is independent of the presence or absence of an α,β-unsaturated carbonyl moiety. It thus appears that because of the great diversity of structural features seen among this group of secondary metabolites, other mechanisms of action could well be involved.

If we now turn our attention to the toxicity of *A. maritima* to snails, it is not yet clear to what extent the sesquiterpene lactones of the plant and the α-methylene-γ-lactone ring are responsible for the molluscicidal activity. Of course, less is still known about the mode of action of these compounds as molluscicides. This led us to conclude that much more studies have to be performed to render the problem clearer.

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using native African plants »
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Royal Academy of Overseas Sciences (Brussels)
pp. 89-100 (1992)

THE TOXICITY OF *AMBROSIA MARITIMA* TO SNAILS AND NON-TARGET ORGANISMS

BY

S. GEERTS*, F. ALARD*, J. BELOT** & M. SIDHOM***

SUMMARY. — The molluscicidal activity of *Ambrosia maritima* is mainly caused by the sesquiterpene lactones present in its leaves, flowers and seeds. Plant populations from different origin show a considerable variation in the level of active principles depending on genetic and environmental factors. Striking differences in the molluscicidal activity of *A. maritima* were observed in field trials in Senegal and Egypt. *A. maritima* is virtually not toxic to non-target organisms: rats, rabbits, algae and *Daphnia*. Sensitization did occur, however, in guinea-pigs and some toxicity was observed in fish at concentrations much higher than those used for snail control.

RÉSUMÉ. — *La toxicité d'*Ambrosia maritima *vis-à-vis des mollusques et des organismes non-cibles.* — L'activité molluscicide d'*Ambrosia maritima* est principalement causée par les lactones sesquiterpéniques, présents dans les feuilles, les fleurs et les akènes. Le taux de principes actifs dans des plantes d'origine différente varie considérablement et dépend de plusieurs facteurs génétiques et écologiques. Le pouvoir molluscicide d'*A. maritima* était fort différent lors d'essais sur le terrain en Egypte et au Sénégal. La plante n'est virtuellement pas toxique vis-à-vis des organismes non-cibles comme le rat, le lapin, les algues et les *Daphnia*. *A. maritima* provoque une sensibilisation chez les cobayes et une certaine toxicité est observée chez les poissons à des concentrations nettement plus élevées que les concentrations molluscicides.

SAMENVATTING. — *De toxiciteit van* Ambrosia maritima *voor slakken en non-target organismen.* — De molluscicide aktiviteit van *Ambrosia maritima* wordt hoofdzakelijk veroorzaakt door de sesquiterpene lactonen, die aanwezig zijn in de bladeren, de bloemen en de zaden. Het gehalte aan aktieve bestanddelen varieert zeer sterk naargelang de afkomst van de plant en wordt bepaald door een aantal genetische

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en omgevingsfaktoren. Sterke verschillen in de molluscicide aktiviteit van *A. maritima* werden waargenomen tijdens terreinproeven in Egypte en Senegal. *A. maritima* is virtueel niet toxisch voor non-target organismen zoals ratten, konijnen, algen en *Daphnia*. Wel werd een sensibilizerend effekt vastgesteld bij cavia's alsook een zekere toxiciteit bij vissen, zij het dan bij veel hogere concentraties dan deze die gebruikt worden voor de slakkenkontrole.

* * *

The molluscicidal activity of *Ambrosia maritima* has been discovered in Egypt. The plant is commonly used in traditional medicine in this country and is known as damsissa (KLOOS *et al.* 1982). The first reports on the activity of *A. maritima* against snails and on the virtual absence of any effects on non-target organisms date from 1962 (SHERIF & EL-SAWY 1962). It is only in the eighties, however, that more detailed research has been carried out in order to evaluate the activity on snails in countries other than Egypt and to study the effects on non-target organisms.

1. Molluscicidal activity of *Ambrosia maritima*

According to SHERIF & EL-SAWY (1962) only the leaves and the flowering parts of *A. maritima* possess a molluscicidal activity. These authors could not detect any effect on snails using the stem or the roots of this plant. The leaves, flowers and seeds of *A. maritima* contain sesquiterpene lactones, secondary metabolites of the plant, volatile and fixed oils, resins, saponins, tannins and other inert substances (FAHMY & DARWISH 1949).The molluscicidal activity of the sesquiterpene lactones, especially ambrosin and damsin, has been proved by different authors (SHOEB & EL-EMAM, 1976; GEERTS *et al.* 1991). The amount of these lactones in the dried leaves, however, varies from almost nihil up to 2.2% (TRIEST *et al.* 1992). Such a considerable variation in the level of secondary metabolites might depend a.o. on the genetic variability, the geographic area, the temperature, the amount of rainfall, the time of collection, etc. of the plant populations examined (FARNSWORTH *et al.* 1987). The most important sesquiterpene lactones, present in *A. maritima*, are ambrosin and damsin. JAPUKOVIC *et al.* (1987) calculated that they represent respectively about 20 and 40% of the total amount of these compounds in the leaves of the plant. Recent analyses of *A. maritima* plants from different geographical origin showed, however, that the amount of damsin and ambrosin may vary considerably and that the quantity of ambrosin is sometimes higher than that of damsin (TRIEST *et al.* 1992). More than 20 different sesquiterpene lactones, the majority being pseudoguaianolides, have been identified in *A. maritima* (SALAM *et al.* 1984; PICKMAN *et al.* 1986; JAPUKOVIC *et al.* 1987; SLACANIN *et al.* 1988; ALI *et al.* 1989). Although sesquiterpene lactones other than damsin and ambrosin were shown to possess molluscicidal activity (GEERTS *et al.* 1991), most of them have not been evaluated. Other compounds (tannins, saponins) present in the plant are known to have some molluscicidal properties (HOSTETTMAN *et al.* 1987) and might produce a synergistic effect, but this has still to be proved. In most of the laboratory experiments dried and powdered leaves of *A. maritima* were used. Therefore the figures cited further in the text all relate to dried plant material. It is not necessary, however, to dry the plant before using it, since fresh green *A. maritima* is able to kill snails as well (SHERIF & EL-SAWY 1962; VASSILIADES & DIAW 1980).

Sesquiterpene lactones are known to have a low solubility in water. Therefore better and more reliable and reproducible results could be obtained using a long exposure laboratory test (4 days) than using a short exposure test during 24 hours (SIDHOM & GEERTS 1984). Alcoholic extracts of *A. maritima* are better soluble in water and consequently significantly lower LC_{50}-values are observed than by using powdered leaves of the plant (Table 1). Concerning the fate of the sesquiterpene lactones dissolved in water only very few

Table 1

Molluscicidal effect of Ambrosia maritima *on snails of Senegalese origin*
(BELOT *et al.* 1991)

A. maritima (dried leaves)	LC_{50} (mg/l)	
	powder	ethanol extract *
Bulinus forskalii	165	62
Bulinus globosus	149	not done
Biomphalaria pfeifferi	227	87
Lymnaea natalensis	108	42

LC_{50}: Lethal concentration for 50% of the molluscs in a long exposure test (4 days).
* Figures expressed as the equivalent of powder from dried leaves.

information is available. SLACANIN *et al.* (1988) observed the complete disappearance of the four most important sesquiterpene lactones in a weakly alkaline aqueous extract after 7 days. Further research in this field is absolutely necessary in order to understand the contradictory results obtained with *A. maritima* under different field conditions.

The susceptibility to the molluscicidal effect of *A. maritima* varies according to the species of snail. Using a single batch of *A. maritima* under laboratory conditions SIDHOM & GEERTS (1984) showed significant differences in susceptibility between *Lymnaea truncatula* and *Biomphalaria glabrata.* BELOT *et al.* (1991) tested 4 different snail species using the same batch of *A. maritima*, the results of which are presented in Table 1.

As has been shown for other molluscicidal plants, there is an important variation of the molluscicidal activity of different *A. maritima* populations (GEERTS *et al.* 1991). Table 2 summarizes the LC_{50} and LC_{95}-values obtained with *A. maritima* from Egypt, Senegal, Ivory Coast and South Africa. Even within one country, e.g. Senegal, the molluscicidal potency (LC_{95}) of some plant populations is 2 to about 4 times higher than other ones, according to the place and the time of harvest (dry or wet season) (BELOT *et al.* 1986). The average molluscicidal activity of the Egyptian populations of *A. maritima* is somewhat higher (lower LC_{95}) than the Senegalese ones, but the difference is not statistically significant. Widely different LC_{50}-values (67 and 222 mg/l) have also been obtained when using plant populations from South Africa (Table 2). These are in correlation with the amount of sesquiterpene lactones present in these plants (TRIEST *et al.* 1992). Sometimes, however, this correlation between active principles and molluscicidal activity is not present. BELOT *et al.* (1992 a) examined 3 batches of *A. maritima* of Senegalese origin. Although all batches contained a similar amount of sesquiterpene lactones, one of the batches showed a much higher molluscicidal effect (LC_{50}: 113 mg/l) than the other two (LC_{50}: 209-274 mg/l). Possible explanations for these contradictory results might be a.o. the presence of molluscicidal principles other than sesquiterpene lactones which produce a synergistic effect and/or the heterogeneity of the results obtained in the molluscicidal tests in the laboratory.

An important feature, observed by BELOT *et al.* (1989), is the fact that a plant population with a high molluscicidal activity does not loose this potency in the subsequent generations (at least until F4)

Table 2

Molluscicidal activity of Ambrosia maritima *from different geographical origin for* Biomphalaria glabrata *(4 day exposure test in the laboratory)* (GEERTS *et al.* 1991)

	Origin of *A. maritima*			
	Senegal	Egypt	Ivory Coast	South Africa
No. of plant populations tested *	7	9	1	2
Mean $LC_{50} \pm SD$ (mg/l) (range)	126 ± 54	91 ± 29	81	145 (67-222)
Mean $LC_{95} \pm SD$ (mg/l) (range)	231 ± 69	195 ± 130	288	252 (121-382)

* Plant populations from different regions within the country or collected during different seasons.

grown in an other environment. This makes it possible—after the selection of a population of *A. maritima* with a high molluscicidal activity—to grow this in endemic regions for schistosomiasis, where snail control has to be carried out. Since the plant can also be grown from cuttings (TRIEST *et al.* 1992), subsequent generations will keep the same characteristics of the parent generation.

Contrary to *Phytolacca dodecandra*, *A. maritima* is able to kill the eggs of the snails (SHERIF & EL-SAWY 1962; SIDHOM & GEERTS 1984). Recent experiments have confirmed this ovicidal effect of the leaves and flowering parts of *A. maritima* of Egyptian origin (GEERTS, unpubl. observ.). Repeated tests using a large number of eggs of *B. glabrata* showed, however, that the susceptibility to *A. maritima* decreased with the age of the eggs. Hundred percent of snail eggs less than one day old were killed at 400 mg/l of the dried plant material, whereas only 57.7 % of 4 day old eggs did not hatch at the same concentration (Table 3).

Concerning the effect of storage on the molluscicidal potency of the plant, contradictory results have been reported (SHERIF & EL-SAWY 1962; SIDHOM & GEERTS 1984). Recently, however, BELOT *et al.* (1992 b) could not prove any clear-cut loss in the molluscicidal activity of *A. maritima* over a period of 20 months.

The most striking phenomenon in relation to the molluscicidal activity of *A. maritima* is its surprisingly high activity in field

Table 3

Ovicidal effect of A.maritima[1] *on the eggs of* Biomphalaria glabrata

	Age of snail eggs (days)					
	< 1		1-2		4	
	C	T	C	T	C	T
No. of egg masses[2] tested	13	13	24	41	13	27
Ovicidal effect (%)[3]						
+ average	11.9	100	7.6	92.2	3.8	57.7
+ S.D.	8.3	0	6.9	12.4	3.3	26.6

C: control; T: treated.
1. *A. maritima*:stored during 2 years before use (origin: Egypt);
2. egg masses: contained each between 20 and 50 eggs, which were individually checked;
3. ovicidal effect: was evaluated after exposure to *A. maritima* powdered plant material at 400 mg/l during 4 days and an observation period of max. 10 days.

experiments in Egypt when compared with the data obtained in the laboratory. All the published reports on field trials by the Egyptian authors (SHERIF & EL-SAWY 1977; EL-SAWY *et al.* 1978, 1981; EL-MAGDOUB *et al.* 1980; EL-SAWY *et al.* 1984, 1987) mention consistently the same excellent results (snail reductions of 90% or more over a period of several months) at a concentration of 35-70 mg/l, which is situated at or below the lower confidence limit (95%) of the average LC_{95} value of the Egyptian plants for *B. glabrata* in the laboratory (195 ± 130 mg/l). Unfortunately no laboratory data are available about the susceptibility of local Egyptian snails to *A. maritima.* Recent preliminary results, however, showed that laboratory reared *B. alexandrina* of Egyptian origin were not significantly more susceptible to *A. maritima* (LC_{50} and LC_{95} resp. 38 and 141 mg/l) than the reference snails *B. glabrata* (GEERTS *et al.*, unpubl. observations). *B. alexandrina* is one of the snail species which was present in most of the field experiments carried out by the aforementioned Egyptian authors. The promising results obtained in the Egyptian experiments could not be confirmed, however, by field experiments carried out in Senegal (BELOT *et al.* 1992 a). In this country *A. maritima*, used at concentrations of 150-300 mg/l in an irrigation canal, resulted in reductions of *B. pfeifferi* by 65.5 to 77% two weeks after treatment. This figure corresponds quite well with the mean LC_{95}-value (418 mg/l; range: 243-523 mg/l) observed in the laboratory using the same batches of *A. maritima* and the same snail species as in the field. Unfortunately no analysis of sesquiterpene lactones was carried out on the plant material used in the Egyptian field experiments in order to allow a comparison with the plants used in Senegal. Further research is absolutely necessary to elucidate the discrepancy between the results obtained in the laboratory and those obtained in the field tests in Egypt and Senegal. A field trial carried out in a third country under well controlled conditions might provide some interesting information. Before going again to the field, however, more data are needed about the solubility and the biodegradation of the sesquiterpene lactones in water and also about the influence of various physico-chemical parameters on the molluscicidal activity of the plant. The mechanism of action of the active principles of *A. maritima* is also an area for further research.

2. Effects of *A. maritima* on non-target organisms

One of the important characteristics of *A. maritima* is its virtual lack of toxicity to organisms other than snails. This phenomenon was observed already in the first Egyptian publications (SHERIF & EL-SAWY 1962), which mention no toxic effects on cattle and sheep eating the plant neither on fish (*Tilapia nilotica*) nor on mosquito larvae exposed to *A. maritima* solutions of 1,000 mg/l. Only recently, however, more precise data (EC_{50} or LC_{50} values) have become available concerning the toxic effects of *A. maritima* on different non-target organisms. The results, which are summarized in Table 4, were obtained using *A. maritima* plants with a known content of sesquiterpene lactones. No acute toxic effects at all could be observed in rats receiving a single oral dose of 5 g/kg of *A. maritima* leaves as powder or as an alcoholic extract. Similarly up to 50,000 ppm of *A. maritima* incorporated into the feed of rats during 4 weeks (semichronic toxicity test) did not result in any toxicity at all (ALARD *et al.* 1991 b). Furthermore, primary dermal and eye irritation tests, carried out using *A. maritima* powdered leaves according to the OECD guidelines, showed that only a very slight to slight irritation could be observed respectively on the skin and in the eye of the exposed rabbits (CROLS 1991). Since many *Ambrosia* species are known as ragweeds or sneezeweeds, possible allergic effects due to *A. maritima* were examined by the Magnusson guinea-pig maximization test. All animals (20) used in the test were strongly sensitized, which proves that *A. maritima* might be able to induce allergic contact dermatitis (CROLS 1991). This observation is not surprising, since ambrosin and damsin contain an alfa-methylene-gamma-lactone moiety which has been proved to be responsible for allergenic effects (MITCHELL 1975). However, none of the authors of this paper, neither the technicians, working in their laboratories and regularly exposed to *A. maritima* (by manipulating and grinding the plants), have experienced any allergic problems.

No mutagenic effects could be observed in the Ames test (with or without metabolic activation) using pure ambrosin or aqueous extracts of *A. maritima* leaves (ALARD *et al.* 1991 b).

The ecotoxicity of *A. maritima* is also very low. ALARD *et al.* (1991 a) showed that toxic effects on algae (*Selenastrum capricornutum*) and crustacea (*Daphnia magna*) were almost absent. Some toxicity was observed, however, when testing fries or juveniles of *Lebistes reticulatus* and *Tilapia aurea.* Mortality in fish occurred only at concentrations which were much higher than the molluscicidal concentrations. Concerning the effects of *A. maritima* on insects rather contradictory reports have been published (SHERIF & EL-SAWY 1962; EL-SAWY *et al.* 1986). VAN BLERK (1991), however, proved that the larvicidal effect of *A. maritima* even at doses as high as 2 000 mg/l was negligible ($< 8\%$) to *Anopheles stephensi* and *Aedes aegypti*. No inhibition of the development of the larvae into adult mosquitoes could be observed at this concentration.

When the toxicity data for *A. maritima* are compared with those for *P. dodecandra* (LAMBERT *et al.* 1991) (Table 4), it is clear that *A. maritima* is much less toxic to non-target organisms than *P. dodecandra.* The risk of developing allergic contact dermatitis, however, cannot be excluded for people who are regularly exposed to *A. maritima.* People manipulating *P. dodecandra* dry berries on the other hand should protect their eyes to avoid severe eye irritation (LAMBERT *et al.* 1991). In spite of the important ecotoxicity of *P. dodecandra* (endod), which is comparable to that of the synthetic molluscicide niclosamide, LAMBERT *et al.* (1991) concluded that field trials using endod are now justifiable. Taking into account all available data about the toxicity of *A. maritima*, the same conclusion can certainly be drawn for damsissa. Further research is necessary, however, on the effect on local non-target aquatic organisms and on the environmental fate when additional field trials are carried out using both *P. dodecandra* and *A. maritima.*

Table 4

Non-Target toxicity of Ambrosia maritima *and* Phytolacca dodecandra

	Parameter	*P. dodecandra**	*A. maritima***
Mammalian toxicity			
Acute oral (rat)	LD_{50} (mg/kg)	1,340	⩾5,000
Semichronic oral (rat)	NOAEL (mg/kg)	500	50,000
Dermal irritation (rabbit)	irritation	slight	very slight
Eye irritation (rabbit)	irritation	severe	slight
Dermal sensitisation (guinea pig)	sensitization	absent	strong
Ecotoxicity			
Algae	EC_{50} (mg/l)	68.5	>1,000
Daphnia	LC_{50} (mg/l)	19.8	>2,000
Fish	LC_{50} (mg/l)	4.4	650
Mutagenicity			
Ames test		non mutagenic	non mutagenic
Sister chromatid exchange		non mutagenic	not done
Snail toxicity			
Field dosage	LC_{90} (mg/l)	35 - 80	35 - 300

* LAMBERT *et al.* 1991
** ALARD *et al.* (1991a & b), CROLS 1991
NOAEL no observed adverse effect level.

Conclusion

From these data it can be concluded that among all molluscicidal plants which have been thoroughly examined, *A. maritima* is certainly the plant with the lowest toxicity to non-target organisms. Some important parameters, however, have received insufficient attention up to now. More information is urgently needed about the solubility and the biodegradation of the sesquiterpene lactones and also about the mechanism of action of these compounds. Once these data are available it would be worthwhile to set up a field experiment to compare *P. dodecandra* and *A. maritima* in the same environment in order to evaluate their effects on snails and snail eggs as well as on non-target organisms.

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COMPARATIVE EVALUATION OF THE MOLLUSCICIDAL ACTIVITY OF *AMBROSIA MARITIMA* IN EGYPT AND SENEGAL

BY

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SUMMARY. — The most important laboratory and field trials using *Ambrosia maritima* in Egypt and Senegal are reviewed. The results are critically analysed. Several factors are discussed which might be responsible for the important differences in the molluscicidal activity of the plant in these two countries.

RÉSUMÉ. — *Comparaison de l'activité molluscicide d'*Ambrosia maritima *en Égypte et au Sénégal.* — Les essais les plus importants de l'action molluscicide d'*Ambrosia maritima* sont passés en revue. Les résultats des expériences au laboratoire et sur le terrain sont analysés de façon critique. Plusieurs facteurs sont discutés, qui pourraient expliquer les résultats contradictoires obtenus dans les deux pays.

SAMENVATTING. — *Vergelijking van de molluscicide aktiviteit van* Ambrosia maritima *in Egypte en Senegal.* — Een overzicht wordt gegeven van de belangrijkste experimenten met *Ambrosia maritima* in Egypte en in Senegal. De resultaten van de laboratorium- en de terreintesten worden kritisch geanalyseerd. Verschillende faktoren worden besproken die verantwoordelijk zouden kunnen zijn voor de uiteenlopende resultaten in beide landen.

* * *

Ambrosia maritima L. (Asteracae) is a herbaceous plant widely distributed over the Mediterranean region and Africa. Its mollusci-

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dal properties are nowadays well known (GEERTS *et al.* 1991). They have been the subject of many studies in the laboratory in Egypt (SHERIF & EIL-SAWY 1962), in Belgium (SIDHOM & GEERTS 1983, 1984; SOMERS 1985) and in Senegal (VASSILIADES & DIAW 1980; VASSILIADES *et al.* 1986; BELOT *et al.* 1986, 1989, 1991).

The sesquiterpene lactones, secondary metabolites of the Compositae, are the molluscicidal components of *Ambrosia maritima.* The content of the sesquiterpene lactones in the plants seems to be different from one country to another, even from one area to another in the same country. Consequently the molluscicidal activity of each natural population of *A. maritima* has to be evaluated before its use in the field (GEERTS *et al.* 1991).

The ability of the plant to control snail populations under field conditions has been studied in Egypt (EL-SAWY *et al.* 1978, 1984, 1987; SHERIF & EL-SAWY 1977; EL-MAGDOUB *et al.* 1980) and in Senegal (VASSILIADES & DIAW 1982; BELOT *et al.* 1992).

The purpose of this paper is to compare the results of a series of experiments, which were carried out in the laboratory and in the field both in Senegal and in Egypt, and to try to understand the factors, which might be responsible for the quite different results obtained in these two countries.

The molluscicidal activity of *A. maritima* in the laboratory

Table 1 summarizes the data on the molluscicidal activity of *A. maritima* obtained in the laboratory after exposure of the snails during 4 days. Important differences in LC_{50}-values are observed according to the snail species and the origin of *A.maritima.* Only for *Biomphalaria glabrata* and *B. pfeifferi* the available data allow a comparison between the molluscicidal effect of *A. maritima* of different origin. These results clearly show that even within one country large variations of the molluscicidal activity of different plant populations do occur. Unfortunately data on the content of sesquiterpene lactones in the tested *A. maritima* material often lack, so that reliable comparisons are difficult. BELOT *et al.* (1992), however, showed that a clear-cut relationship between the amount of sesquiterpene lactones and the molluscicidal potency of the plant is not always present.

Only few data are available on the susceptibility of snails of Egyptian origin to *A. maritima* under laboratory conditions (Table 1).

Table 1

Laboratory evaluation of the molluscicidal effect of Ambrosia maritima *(dried leaves) from Egyptian or Senegalese origin.*

SNAIL		Range of LC_{50}-values ($mg\,l^{-1}$) * using *A. maritima* from	
Species	Origin	Senegal	Egypt
BIOMPHALARIA			
alexandrina	Egypt	32 - 45	N.D.
glabrata	Puerto Rico	42 - 225	47 - 149
pfeifferi	Senegal	99 - 315	201 - 257
BULINUS			
forskalii	Senegal	N.D.	152 - 179
globosus	Senegal	N.D.	143 - 155
LYMNAEA			
natalensis	Senegal	N.D.	101 - 116

* LC_{50} values calculated from long exposure test (4 days). Data compiled from BELOT *et al.* 1991, 1992; GEERTS *et al.* 1991, 1992.
N.D.: not done

SHERIF & EL-SAWY (1962) tested different Egyptian snail species at a concentration of 1,000 $mg\,l^{-1}$, but they did not calculate any LC_{50} values. SHOEB & EL-EMAM (1976) are the only authors, who calculated LC_{50} values for *B. alexandrina* and *B. truncatus* of Egyptian origin using pure ambrosin or damsin. Their results ($LC_{90}{}^{.24h}$: 8.5-10.9 $mg\,l^{-1}$) correspond rather well with those obtained by GEERTS *et al.* (1991) using *B. glabrata* ($LC_{100}{}^{.96h}$: 10 $mg\,l^{-1}$) as far as ambrosin is concerned. Contrary to SHOEB & EL-EMAM (1976) GEERTS *et al.* (1991) used a long exposure test (96 h), but snail mortality was already observed after 24 hours. The molluscicidal activity of damsin, however, was higher (LC_{90}: 9.7-13.5 $mg\,l^{-1}$)in the experiments of SHOEB & EL-EMAM (1976) than in those carried out by GEERTS *et al.* (1991), who observed only 40% mortality at 10 $mg\,l^{-1}$. MARCHANT *et al.* (1984) on the other hand were not able to detect any molluscicidal activity using damsin. These contradictory results might be explained by the fact that damsin is a rather unstable compound. Consequently the duration of storage or other factors might influence the molluscicidal potency.

The molluscicidal activity of A. maritima in the field (Table 2)

The molluscicidal activity of *A. maritima* in the field in Egypt has been tested repeatedly. A first field trial with the whole dry or fresh plant immersed in the water of irrigation canals showed a molluscicidal effect beginning at 7 days after the application of 70 mg l^{-1} and lasting over a period of 16 months (EL-SAWY *et al.* 1978, 1981) . In other better controlled field experiments on a larger scale three concentrations (35, 70 and 140 mg l^{-1}) of the whole dried plant (collected from the wild, region of Aswan) were tried out in a series of irrigation canals and drains, 500 m long, 1-2 m wide and 0-0.5 m deep (EL-SAWY *et al.* 1984). The reduction in the numbers of alive *Biomphalaria alexandrina* snails (over a 3 month period) was generally more than 90% in both types of watercourses and was virtually the same at all treatment levels, even at 35 mg l^{-1}. In a further field test, EL-SAWY *et al.* (1987) compared the molluscicidal activity of fresh and dried *A. maritima* (cultivated plants, Alexandria). The test concentrations were limited to 70 mg l^{-1} of dried leaves (equivalent fresh material: 280 mg l^{-1}) and 140 mg l^{-1} (equivalent fresh material: 560 mg l^{-1}). The fresh plants were used at a concentration four times higher than the dried ones, since it was shown that *A. maritima* loses 75% of its weight after drying. No statistical differences were found between the molluscicidal activity of fresh and dried plant material at these concentrations. The snail reduction two weeks after the application of fresh or dried *A. maritima* was respectively 72 and 77%, taking into account the evolution of the snail population in the control canals. The molluscicidal effect was more pronounced in the period from 2 weeks until 2 to 3 months post treatment. This remanent effect in the treated watercourses was explained to be caused both by the molluscicidal application and by a natural fall of the snail oviposition during the months that followed the treatments. The population of snails persisted, however, at a low level in the canals and drains during the whole observation period. The authors (EL-SAWY *et al.* 1984; 1987) were not able to confirm the long lasting molluscicidal effect (16 months) as reported earlier (EL-SAWY *et al.* 1981).

In Senegal a first field experiment was carried out in a pond (VASSILIADES & DIAW 1982). Two successive trials were carried out using dried leaves of *A. maritima* (wildly growing plants, region of Thiès), which were not immersed but powdered on the surface of the

Table 2

Description of the main molluscicidal field trials using Ambrosia maritima *in Egypt and Senegal*

Waterbodies	Volume of water	Number of tests	Concentration of *A. maritima* (dried plant material) $mg \cdot l^{-1}$	Molluscicidal effect	
				snail species	% reduction
A. *Egypt*					
1. Alexandria[1]	± 225 m^3	4	35	*B. alexandrina*	94 *
— irrigation canals		2	70		
(waterflow:		1	140		
negligible)		4	0 (control)		
— drains	± 225 m^3	4	35	*B. alexandrina*	89 *
		2	70		
		4	140		
		5	0 (control)		
2. Alexandria[2]					
— irrigation canals and drains	± 225 m^3	6	70	*B. alexandrina*	77 **
		3	140		
		6	0 (control)		
B. *Senegal*					
1. Niayes, Dakar[3]	5 - 13 m^3	2	375 to 400	*Lymnaea natalensis*	89-96 ***
— pond			(no control)		
2. Lampsar[4]	50 m^3	1	200	*Bulinus* spp.	0 ***
— creek		2	400		54-56 ***
		1	0 (control)		
3. Richard Toll[4]	242 m^3	1	150	*Biomphalaria pfeifferi*	77 ***
— irrigation canal		1	300		65.5 ***
off irrigation		1	0 (control)		
— open canal	242 m^3	1	300		0 ***
(waterflow: 0.4 m/s)		1	0 (control)		

1. El Sawy *et al.* (1984).
2. El Sawy *et al.* (1987).
3. Vassiliades & Diaw (1982).
4. Belot *et al.* (1992).

* Average for the 3 dosages combined and over a 3 month period from 1 to 4 months post treatment.

** Average for the 2 dosages combined two weeks after treatment.

*** Two weeks after treatment.

water at a concentration of 375 to 400 mg l^{-1}. One week after the application more than 90 % of the population of *Lymnaea natalensis* was killed. The snail mortality was less pronounced two weeks after treatment due to the presence of a lot of young living snails. The authors explained this by the lack of ovicidal activity of *A. maritima* and concluded that snail control using *A. maritima* was not very practical due to the large quantity of *A. maritima* they needed for the treatment of a small pond.

In order to evaluate if these high concentrations of *A. maritima*, as described by VASSILIADES & DIAW (1982), were really necessary to control snails under Senegalese conditions, three successive tests were carried out in the delta of the Senegal River using a native population of *Ambrosia maritima* (Mboro, 80 km from Dakar) (BELOT *et al.* 1992). Before each treatment, the batch of *A. maritima* used in the field, was tested in the laboratory to determine its content of sesquiterpene lactones and to evaluate its molluscicidal activity against *Biomphalaria pfeifferi*. The three batches contained a similar amount of sesquiterpene lactones (3-4 % of the dry matter of the leaves) but their molluscicidal activity varied considerably (LC_{50} :113-274 mg l^{-1}; LC_{95}: 243-523 mg l-1). The molluscicidal applications were carried out at two different sites in the delta of the Senegal River. The first trial was done at Lampsar, a village along a «marigot» supplied with water by the Lampsar River. Along the banks of the «marigot» creeks are formed which are separated from the marigot by plant fences and floating islands. A creek with a volume of 50 m^3 of water and a surface of 61.5 m^2 was chosen for the molluscicidal applications, whereas another creek with the same proportions was kept as the negative control. Three successive experiments were done using fragmented dried leaves of *A. maritima* which were immersed in the creek at final concentrations of 200 mg l^{-1} (first experiment) and 400 mg l^{-1} (second and third experiment). The bags containing the leaves of *A. maritima* were made to sink in the creek (depth : maximum 1.5 m) by adding stones and kept in the water during 28 days. At the end of each experiment the bags were removed and examined. The snail surveys were carried out 1, 2 and 4 weeks after the immersion of the plant material. No statistically significant decrease in the population of *Bulinus* spp could be observed in the treated creek at 200 mg l^{-1}. Reduction rates of about 50 % were present two weeks after treatment with 400 mg l^{-1}, but the molluscicidal effect had disappeared completely 4 weeks post treatment.

The second field trial was carried out in an irrigation canal in a sugar cane field at Richard Toll (Compagnie Sucrière Sénégalaise). This irrigation canal was 7 m wide and 1-1.65 m deep. A section was divided into three parts, each of 25 m, which served respectively as treated, buffer and control parts. The volume to be treated was carefully calculated to 242 m^3. Three successive applications of *A. maritima* were carried out: the first and the second at respectively 150 and 300 mg l^{-1} while the canal was not used for any irrigation purposes, i.e. in stagnant water; the last one at 300 mg l-1 under normal conditions of irrigation at a maximum waterflow of 0.4 m/s.

One and 2 weeks after the molluscicidal applications (150 and 300 mg l^{-1}) in stagnant water (canal off irrigation) a significant decrease of the density of *B. pfeifferi* was observed in the treated part. Snail reduction, however, never exceeded 77% of the initial population, taking into account the evolution of the snails in the control section. When the canal was used again for irrigation purposes two weeks after treatment, a fast increase of the snail density was observed. No snail reduction at all was achieved when *A. maritima* was applied at 300 mg l^{-1} in flowing water.

When the bags were examined 2 and 4 weeks after the start of the experiments, the leaves of *A. maritima* were clearly in a stage of decomposition. At the surface they were covered with algae and healthy snails (*Bulinus* and *Biomphalaria*) were present on them (BELOT *et al.* 1992).

Discussion and conclusions

From the experiments in Senegal it is clear that *Ambrosia maritima* had little or no effect in flowing water or in a volume of water (creek) in contact with a larger water body. The sesquiterpene lactones, which are slowly released from the plants, are probably removed by the waterflow and cannot reach a sufficiently high concentration to kill the snails. The only significant reduction of the snail population has been observed in stagnant water during 15 days after the immersion of *A. maritima* at 15 up to 300 mg l^{-1}. Although the observations were stopped or could not be continued under stagnant water conditions, there are strong indications for the absence of a remanent molluscicidal effect (VASSILIADES & DIAW 1982; BELOT *et al.* 1992).

These results are in sharp contrast with the results obtained in Egypt (EL-SAWY *et al.* 1984), where higher snail reductions (89 to 94 %) were observed at lower concentrations of *A. maritima* (35-70 mg l^{-1}). It must be noticed, however, that these figures cover a period from approximately 1 to 4 months post treatment. In a further experiment, where the snail population was examined 2 weeks after treatment, snail reduction was less pronounced (EL-SAWY *et al.* 1987). An average decrease of only 77 % was observed for the 2 dosages (70 and 140 mg l^{-1}) of dried *A. maritima* which were used. Exactly the same reduction (77 %) was reported by BELOT *et al.* (1992) in the Senegalese irrigation canal treated at 150 mg l^{-1}. After treatment at 300 mg l^{-1} , however, the reduction rates were 74.8 and 65.5 % at respectively 1 and 2 weeks post treatment.

It is very difficult to compare the field trials in Egypt with those in Senegal, because they have been carried out under completely different conditions. At least 3 important parameters are different in the 2 countries: 1. the batch of *A. maritima* used; 2. the physico-chemical parameters of the water and 3. the target snail species.

Concerning the *A. maritima* material which was used in Egypt and Senegal, the content of sesquiterpene lactones is only known for the plants used in the experiments carried out by BELOT *et al.* (1992) in the latter country. This makes any reliable comparison between the trials impossible. Since the molluscicidal activity of different plant populations within one country can vary considerably, it cannot be excluded that the *A. maritima* used in the Egyptian trials might contain a particularly high amount of sesquiterpene lactones, but this is rather unlikely, because analyses of different Egyptian *A. maritima* plants showed similar amounts of active principles as the Senegalese ones (TRIEST *et al.* 1992).

Contrary to the Senegalese trials, no data are available about the physicochemical parameters of the water in the Egyptian field experiments. It is known that temperature, pH, turbidity, conductivity and salinity of the water have an important effect on the activity of chemical or plant molluscicides. Therefore it is possible that these parameters might have been more favourable in Egypt than in Senegal, which could explain the better results in the former country. Because of the shallow water it is likely that the temperature in the Egyptian irrigation canals and drains might have been higher than that in Senegal. This factor might thus have enhanced the molluscicidal activity of *A. maritima* due to the better solubility of the sesquiterpene lactones in warm water (SHERIF & EL-SAWY 1962), but it is questionable if this alone could explain the much better results in Egypt.

Finally the target snail species in the various field trials were also different. The available data mainly concern *B. alexandrina* in Egypt and *L. natalensis* and *B. pfeifferi* in Senegal. Although *L. natalensis* seems to be more susceptible to the molluscicidal effect of *A. maritima* than *B. pfeifferi* in the laboratory (BELOT *et al.* 1991), this is not directly evident from the field trials in Senegal. In the pond, populated by *L. natalensis* (VASSILIADES & DIAW 1982), *A. maritima* caused a higher snail reduction than in the canal, infested by *B. pfeifferi*, but the plant concentration was also higher in the former trial (375-400 mg l^{-1}) than in the latter (150-300 mg l^{-1}). Unfortunately VASSILIADES & DIAW (1982) did not evaluate lower concentrations of *A. maritima*. Furthermore the plant material was not completely immersed in the pond trial, which also compromises any comparison of both Senegalese trials. A direct comparison of the susceptibility of the Egyptian *B. alexandrina* and the Senegalese *B. pfeifferi* using the same batch of *A. maritima* has not been done until now. The available data seem to indicate, however, that Senegalese *B. pfeifferi* are more resistant than Egyptian *B. alexandrina* (GEERTS *et al.* 1992). When both species are compared with *B. glabrata*, as a reference, the LC_{50}-values for *B. pfeifferi* (113-274 mg l^{-1}) are consistently higher than the average LC_{50} value (91 mg l^{-1}) for *B. glabrata*, whereas the LC_{50}-value for *B. alexandrina* is lower (32-45 mg l^{-1}).

Although the above-mentioned parameters do not allow an objective comparison of the field trials in Egypt and Senegal, some general remarks can be put forward. Firstly it is very striking that in **all** the Egyptian field trials *A. maritima* gave snail reduction rates of $>90\%$ at 70 mg l^{-1} or less, whereas a laboratory evaluation of 9 different *A. maritima* populations of Egyptian origin showed widely different LC_{95} values (195 ± 130 mg l^{-1}) (GEERTS *et al.* 1992). One should expect at least one experiment in which less good results were obtained. In Senegal the molluscicidal concentrations used in the field trials (150-400 mg l^{-1}) correspond much better with the LC_{95}-values obtained in the laboratory using *B. glabrata* (231 ± 69 mg l-1) or using *B. pfeifferi* (243-523 mg l^{-1}).

A second remark is the absence of a real dose-effect relationship in the field trials in Egypt as well as in Senegal. EL-SAWY *et al.* (1984)

mention virtually the same molluscicidal effect at 3 treatment levels (35, 70 and 140 mg l^{-1}), whereas BELOT *et al.* (1992) could not show any significantly different effect after treating at 150 and 300 mg l^{-1}. It is very surprising that *A. maritima* concentrations 2 or 4 times higher than the lowest concentration do not produce significantly better results. This aspect needs to be elucidated. Therefore it would be relevant to analyse further the data obtained by EL-SAWY *et al.* (1984) in order to evaluate the variation of the molluscicidal effect obtained in the different canals and drains at the various treatment levels. Unfortunately only means and no standard deviations of snail counts are mentioned in their publication.

Furthermore the results of the experiments in Egypt and Senegal have to be interpreted cautiously, since the presentation of the data is quite different. The highest reduction rates in the Egyptian experiments are obtained when the figures are combined over a longer period (3 months) after treatment. It cannot be excluded, however, that other factors such as a natural decline in the snail population during this period might have enhanced the effect caused by *A. maritima.* When the results are compared at an earlier time after treatment (2 weeks), the differences between the Egyptian and the Senegalese experiments are much less pronounced: an average reduction of 77% at 70 to 140 mg l^{-1} in the former versus 71% (65.5-77) at 150 to 300 mg l^{-1} of *A. maritima* in the latter.

From all these observations it can be concluded that more experiments are necessary under well controlled conditions and using identical standard protocols in order to confirm either the promising results obtained in Egypt or the less promising results from Senegal.

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