SESSION 3A

LINKING ENVIRONMENTAL FATE AND NON-TARGET EFFECTS OF PESTICIDES: AQUATIC ECOSYSTEMS

Chairman	Dr T Roberts *JSC International Ltd, Harrogate*
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Papers	3A-1 to 3A-4

THE USE OF HIGHER TIER MESOCOSM STUDIES IN AQUATIC RISK ASSESSMENT UNDER 91/414/EEC: A UK REGULATOR'S PERSPECTIVE

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ABSTRACT

Under the EC Plant Protection Product Directive 91/414/EEC, higher tier micro-/mesocosm studies are required based on TER triggers from the initial aquatic risk assessment. Their main purpose is to refine the aquatic risk assessment by bringing together more realistic data on environmental fate and effects processes. However, in order to make more efficient use of mesocosm studies and to avoid the type of data interpretation problems formerly encountered by the USA EPA, it is important to first consider exactly what the mesocosm needs to address and then design the study accordingly. The key regulatory issues associated with mesocosm studies are discussed.

INTRODUCTION

The EC Plant Protection Product Directive 91/414/EEC (Council Directive, 1991) sets out in Section 8.2 of Annexes II and Section 10.2 of Annex III, an aquatic toxicity testing strategy for pesticide active ingredients and pesticide products respectively (Council Directive, 1996). This testing strategy adopted by the EC is a tiered approach, which starts with acute laboratory based toxicity studies on indicator species of fish, aquatic invertebrates and algae/plants. The second tier of testing which is determined by the pattern of exposure from the use of a pesticide, involves longer term laboratory based chronic testing on fish and aquatic invertebrates. The final tier of testing for aquatic effects involves micro/mesocosm type studies, and the requirement for such studies is determined by carrying out a risk assessment. This paper presents an interpretation of the potential role of micro/mesocosm studies in aquatic risk assessment under 91/414/EEC. In particular, this paper will investigate the regulatory requirement, design, function, use and limitations of micro/mesocosm studies.

Although separate definitions exist for both mesocosm and microcosm studies, which are largely based on size, for the purpose of this paper no distinction has been made between micro- and mesocosm studies since they are both considered to be higher tier studies which are designed to investigate effects on aquatic life under more realistic conditions.

AQUATIC RISK ASSESSMENT UNDER 91/414/EEC

The procedures for aquatic risk assessment under 91/414/EEC are reported under the relevant sections of Annex III (Council Directive, 1996), which contain the product based data requirements and Annex VI (Council Directive, 1994), which contain the 'Uniform

Principles' for mutual acceptance. The aquatic risk assessment which underlies these Annexes is the calculation of toxicity : exposure ratios (TERs). This is a comparison of the available aquatic effects data in the form of LC/EC50s or NOECs, with predicted environmental concentrations (PECs) estimated from the pattern of use and environmental fate and behaviour characteristics of a pesticide. The main route of exposure considered when assessing the risk to the aquatic environment from pesticide use is currently spray drift, which is estimated using the spray drift deposition data generated by the German BBA (Ganzelmeier *et al.*, 1995). Although spray drift based risk assessment is the predominant scenario currently assessed, other routes of surface water contamination such as leaching/drainage and run-off are also considered where relevant (e.g. pre-emergence uses of mobile pesticides). For guidance on how to conduct an aquatic risk assessment Annex III of 91/414/EEC refers to the European and Mediterranean Plant Protection Organisation and Council of Europe (EPPO/CoE) decision-making schemes (EPPO/CoE, 1994). This EPPO/CoE aquatic risk assessment scheme, like the Annex II (active substance data requirements) and III data requirements, follow a sequential or tiered approach based on predicted exposure, calculation of TERs, comparison of TERs with empirically derived trigger values and, importantly, the use of expert judgement.

REQUIREMENT FOR MESOCOSM TESTING

The key trigger values, within 91/414/EEC, which may require mesocosm testing are reported in Annex III and VI, and are currently an acute TER of ≤100 for fish or aquatic invertebrates (based on LC/EC50), a TER of ≤10 for algae (based on EC50) and a chronic TER of ≤10 for fish or aquatic invertebrates (based on NOEC). However, it is important to note that these Annex III TER triggers require expert judgement to be used first before deciding whether a mesocosm study is required. Such expert judgement should take into consideration aspects such as the environmental fate and behaviour profile of the active substance (e.g. partitioning of active substance to sediment or degradation in water), the nature of effects on aquatic life (e.g. algistatic versus algitoxic effects) and the pattern of use of a pesticide (e.g. single applications versus multiple applications), all of which may influence the predicted risk to aquatic life.

If, after using expert judgement, a 'refined' TER is still below the appropriate Annex III & VI threshold values and the predicted risk is considered to be unacceptable, the risk to aquatic life needs to be assessed further. However, there are two more important points which should be considered before deciding whether a mesocosm study is appropriate. These are as follows:-

(i) Can it be predicted from the available laboratory data on the toxicity, physico-chemical properties and environmental fate and behaviour of a pesticide, that the predicted risk based on TER calculations is highly likely to be realised in a mesocosm study?

Where based on the available data a mesocosm study is likely to confirm the results of the TER based assessment, there is little point in conducting such a study and instead it may be more appropriate to consider risk management options. For example, where the acute TERs for fish are very low (e.g. TER <1) and the pesticide active substance is not readily degraded or adsorbed to sediment, a mesocosm study is likely only to confirm the TER predicted high acute risk to fish. Alternatively, where the available laboratory data indicate that the risk to aquatic life under more realistic field conditions (e.g. mesocosm study) may be less than that predicted using the TER approach, it is appropriate to conduct a mesocosm study. For example, where an acute risk to fish is identified from the TER assessment and the pesticide active substance is reported to be either rapidly hydrolysed in water (e.g. DT50 < 2 days) or rapidly adsorbed to sediment, it would be appropriate to conduct a mesocosm study, since such a study may show a lower risk to fish due to reduced exposure under more realistic field conditions.

(ii) Is there a risk management option available which is both acceptable to the registrant and which will reduce the risk to aquatic life such that the Annex III & VI threshold values can be met?

Where a risk to aquatic life is identified from a TER assessment and there is a risk management option available (e.g. no-spray zone), which is both acceptable to the registrant and offers sufficient protection to aquatic life in order to satisfy the regulatory authority, then a mesocosm study does not need to be conducted.

MICRO-/MESOCOSM DESIGN

Whilst European mesocosm studies conducted in the 1980's used relatively small sized systems (commonly between 1-25m^3), the USA Environmental Protection Agency (EPA) developed and issued in 1988 a regulatory guidance document which recommended that mesocosm studies should be carried out using test systems with a minimum size of 400m^2 (0.1 acre) surface area (Touart, 1988). However, in 1992 the US EPA stopped requesting mesocosm field testing partly due to the difficulty they had in interpreting the results of these very large and complex systems. Mesocosm design was further debated in 1991 at both a USA 'SETAC-RESOLVE' Workshop in Wintergreen, Virginia, and at a 'SETAC-Europe' Workshop held at Monks Wood, Huntingdon, UK. Both these workshops published their proposals, which reached broadly similar conclusions, i.e. that smaller mesocosm or microcosm systems (e.g. 1- $\geq$25m^2), which were designed on a case by case basis to address specific questions, could be used to satisfy regulatory requirements (SETAC-RESOLVE, 1992; SETAC-Europe, 1991). Indeed these conclusions were further endorsed at a later 'European Workshop on Freshwater Field Tests' held in Potsdam, Germany in 1992 (EWOFFT, 1992), which was held in order to discuss the recommendations out of the earlier USA and European workshops. The aforementioned SETAC-Europe Workshop produced a 'Guidance document on testing procedures for pesticides in freshwater mesocosms' (SETAC-Europe, 1991) and this together with the EWOFFT publication (EWOFFT, 1992), are currently considered to offer the best available guidance on mesocosm design and are referenced within the 91/414/EEC Annex III mesocosm study data requirement.

Mesocosm studies are essentially artificial systems which simulate aquatic ecosystems, and offer a compromise between standardised laboratory studies and variable actual ecosystems (Shaw & Kennedy, 1996). Consequently, not only can mesocosms which are conducted for regulatory purposes be conducted in small test systems (sometimes known as microcosms) but, in some situations, studies can also be conducted indoors as well as outdoors. Whilst the relevant aquatic organisms section of Annex VI of 91/414/EEC refers to "under field conditions", this does not necessarily mean that a mesocosm study cannot be conducted indoors. The key element of such a study is to mimic more realistic exposure resulting from outdoor/field use (e.g. presence of sediment, pattern of exposure). Therefore, there is no reason why more realistic field conditions cannot be simulated under controlled laboratory conditions. The following example of an indoor micro-/mesocosm design agreed between PSD and a registrant was used to investigate the risk to aquatic macrophytes from leaching/drainage contamination of water arising from the use of a sulfonyl-urea herbicide:-

A drainage ditch and receiving watercourse monitoring study, following recommended use of a sulfonylurea herbicide, reported levels between 0.01 and 5.02 µg ai/litre in drainage water and between <0.01 and 1.86 µg ai/litre in the receiving watercourse. Information was also available from this study on the duration of each of these contamination events. When these measured exposure levels were compared with laboratory toxicity data, TERs of 0.038 - 19 resulted, which indicated a potential high risk to aquatic macrophytes from leaching/drainage contamination. Consequently, PSD required a mesocosm study to investigate the risk to aquatic macrophytes from this route of exposure. Following consultation between the registrant, testing laboratory and PSD, a protocol for an indoor laboratory mesocosm study was drafted, which mimicked six different exposure patterns reported from the field drainage monitoring study. These were flow-through studies at 0.1 µg ai/litre - 5 days; 0.5 µg ai/litre - 5 days; 1.0 µg ai/litre - 12 hours; 2.0 µg ai/litre - 6 hours; 5.0 µg ai/litre - 3 hours; and a static study at 5.0 µg ai/litre. These exposure patterns, which were tested in the laboratory in glass aquaria containing water and in some cases also pea gravel (representing worst case low adsorptive substrate), were used to assess the effects of the herbicide on 3 different aquatic macrophyte species representing floating, emergent and submersed plants all of which may be present in the natural environment. The results of this study indicated no significant adverse effects on any of the species tested from any of the exposure patterns, and PSD subsequently concluded that leaching/drainage contamination arising from the use of this compound presented a low risk to aquatic macrophytes.

The above mesocosm study is a good example of a 'targeted' small scale indoor study which successfully mimicked more realistic field exposure without actually conducting the study outdoors. This example also demonstrates the importance of making the maximum use of the available data and the benefits of consulting with the regulatory authority during the protocol development stage.

REGULATORY USE OF MESOCOSMS

The initial laboratory based TER risk assessment is based on a realistic worst case scenario, and in general, is also heavily influenced by the laboratory based toxicity data. The main uncertainty surrounding the TER at this stage is the exposure in the form of the 'PEC' and the potential inter-specific variation in sensitivity between the laboratory indicator species and other exposed species in the field. Thus the mesocosm study is the first opportunity to actually directly link more realistic and relevant environmental fate and effects processes.

Thus the main purpose of a mesocosm study is to reduce the level of uncertainty in this laboratory TER based risk assessment by extending the toxic effects information over a wider taxonomic range of relevant species under more realistic simulated field exposure conditions. As such, the mesocosm study has 3 main regulatory uses:-

(i) To enable the registrant to over-rule the risk identified in the TER assessment by demonstrating no adverse effect of the pesticide at levels likely to occur from normal field use.

(ii) Where adverse effects are demonstrated at levels likely to occur from normal field use, the mesocosm study can be used to refine the laboratory based aquatic environment NOEC, which can in turn be used in the development of an appropriate risk management technique.

(iii) Where adverse effects are demonstrated at levels likely to occur from normal field use, the mesocosm study can be used to qualify and quantify the nature of effects likely to occur (with or without risk management restrictions), which in turn will aid assessment of acceptability of such effects.

LIMITATION OF MESOCOSMS

Although, the currently recommended smaller (1-25 m^3) mesocosm study designs (SETAC-Europe, 1991), works well for investigating the risk to algae, plants and aquatic invertebrates, such mesocosms are not ideal for investigating risk to fish. For investigating acute effects on fish, the use of 'cages' or 'enclosures' within such mesocosms, is often recommended for ease of sampling and to prevent grazing of invertebrates (where effects on invertebrates are also being studied in parallel). Whilst, it still is possible to conduct longer term studies investigating growth effects on fish in such mesocosm studies, they are not recommended for investigating reproductive effects in fish (SETAC-Europe, 1991). Instead, laboratory data such as a Fish Life Cycle test should be considered for this purpose (EWOFFT, 1992).

One of the important processes which a higher tier study should monitor is recovery following a toxic effect. For mesocosms, only by selecting a study duration relevant to the test species population demography, can recovery currently be directly measured (Shaw & Kennedy, 1996), and even then this recovery is restricted to that resulting from reproductive/demographic or regenerative processes. However, reproductive/demographic or regenerative potential is only one of the recovery processes operating in the natural environment. The other major recovery process operating in the natural environment, which the mesocosm study because of its virtually 'closed nature' has very limited ability to measure, is recolonisation. Consequently, recovery is currently easier to demonstrate in mesocosms designed to investigate effects on algae and aquatic plants for which reproductive/regenerative processes can dominate. In contrast however, recovery is not easy to demonstrate or measure in mesocosms designed to investigate effects on fish or certain aquatic invertebrates (e.g. crustaceans). In such cases where it is not possible to demonstrate recovery due to the unavailability of seasonal colonisers, then under certain circumstances bioassays can be used to show that biological water or sediment quality has returned to normal (SETAC-Europe, 1991). Alternatively, recovery could be monitored via the introduction and subsequent monitoring of new test organisms at specified time periods after the pesticide application (re-introduction time periods based on the environmental fate and behaviour profile of the pesticide). Although further research is required in order to more fully understand the processes which may affect the rate of recovery of aquatic ecosystems (EWOFFT, 1992), it is important that mesocosm design is eventually improved to better reflect both recolonisation and reproductive/regenerative recovery processes. It should be noted that recovery is already established as an important factor in both the design and interpretation of the terrestrial equivalent of mesocosm studies, i.e. terrestrial invertebrate field studies (e.g. effects on non-target arthropods, earthworms and soil microbial processes).

MESOCOSM USE IN DETERMINING ACCEPTABILITY

Although guidance is now available on how to design mesocosm studies to detect biological effects (SETAC-Europe, 1991), currently there is little, if any, guidance on how to determine the acceptability or otherwise of any reported effects. With regards to assessing 'ecological acceptability' of reported effects in a mesocosm study, the only formal regulatory guidance available is that reported in the current draft of the Uniform Principles (Annex VI) of 91/414/EEC (Council Directive, 1994). Currently, acceptability within the Uniform Principles is defined by either threshold predictive TER values (i.e. acute TER$\leq$100 or chronic TER $\leq$10 for aquatic organisms) or by the following text with regards to effects in the field, which are relevant for assessing mesocosm effects :- "under field conditions no unacceptable impact on the viability of exposed species occurs, directly or indirectly (predators), after use of the product according to the proposed conditions of use" (Council Directive, 1994).

Within the scientific community it is widely accepted that the key factors to be used in determining acceptability of effects are the size and duration of any impact at a population or ecosystem level. Therefore, recovery has an important role in the assessment of acceptability of mesocosm effects. Consequently, as pointed out earlier, more work is required in order to improve the design of mesocosm studies to reflect both the recolonisation and demographic recovery processes which will aid interpretation of acceptability of mesocosm effects. Another factor which is important in assessing acceptability of mesocosm effects is the spatial and temporal scale of effects. For example, a potential population or ecosystem impact as predicted from a mesocosm study may be acceptable if the associated pesticide use (and hence area of risk) is small scale, infrequent and localised. Whereas a lesser but still significant population impact may be unacceptable if associated with a common widespread and intensive pesticide use (e.g. use on cereals). However, other factors such as the conservation importance (e.g. protected or rare species), aesthetic or commercial value (e.g. fish kills) of the aquatic species at risk also need to be considered. Also of concern would be impacts that resulted in ecosystem function parameters being compromised e.g. carbon cycling. However, further research on assessing ecosystem function effects is required in order to more fully understand their part in assessing acceptability of aquatic effects (SETAC-Europe, 1991).

Development of agreed regulatory threshold criteria to indicate what constitutes an unacceptable aquatic effect, is both highly desirable but also highly complicated. Therefore, in the short term, it may be easier to judge ecological unacceptability of aquatic effects by comparison with a toxic or 'positive' standard. This approach has been used successfully in the terrestrial invertebrate field where the broad spectrum organophosphate insecticide, dimethoate, has been used as a toxic standard for non-target arthropod field studies. In this case the toxic standard represents the worst case adverse non-target arthropod impact, which would be considered acceptable, with appropriate use restrictions, from an approved pesticide. In such cases, if the effects of the test compound are more severe than the toxic standard they are considered to be unacceptable and no approval would be authorised. For this approach to work for mesocosm studies an aquatic toxic standard needs to be identified . Similarly, to identify significant adverse aquatic effects which were to be considered acceptable or tolerable without any need for risk management, a 'soft' standard could be used. A soft standard normally will result in significant adverse effects which are more limited in terms of their intensity, duration and/or selectivity of effects, as well as may aid interpretation of indirect effects such as removal of food source. For example, the selective acaricide pirimicarb is often used as the soft standard in non-target arthropod field studies. In such cases, if the effect of the test compound is comparable to the soft standard then it is considered to be acceptable.

Ultimately, however, it should be remembered that acceptability will rarely be considered on aquatic effects alone. Overall regulatory acceptability will be made on a case by case basis, based on risk / benefit analysis, which will consider aquatic risks alongside other wider environmental issues (e.g. comparative risk to terrestrial environment) and cost/benefit issues such as the implications for crop and human safety as well as economic implications to the farmer and consumer. Such wider acceptability issues are currently considered by the Advisory Committee on Pesticides (ACP) in the UK.

CONCLUSION

Smaller scale and targeted micro- or mesocosm studies as described by SETAC have a valuable role to play in regulatory aquatic risk assessment under 91/414/EEC. However initially, great care needs to be exercised in deciding whether it is appropriate or not to conduct a mesocosm study. There is little point in conducting a mesocosm study if either additional laboratory effects or fate data can be better utilised or if the mesocosm study is very likely to merely confirm the original TER risk predictions. Once the decision to conduct a mesocosm study has been taken, in order to make more efficient use of such a mesocosm study, great care also needs to be taken in designing the study protocol to answer the specific questions raised in the predictive TER based risk assessment. In order to improve the regulatory utilisation of mesocosm studies, research is required to produce a more effective mesocosm design for monitoring recovery, which in turn will aid interpretation of acceptability of effects. If recovery cannot be successfully demonstrated in mesocosm studies then the alternative is field monitoring for aquatic effects following practical use. However, this type of data may well suffer from the same data interpretation problems associated with the old USA EPA large pond studies.

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EXPERIMENTAL SIMULATION OF PESTICIDE LOADING IN A STREAM MESOCOSM

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ABSTRACT

An artificial stream mesocosm was treated with a formulated ^{14}C-pesticide in autumn 1995 simulating an overspray event. Water, sediment, algae and fish samples were taken at certain time periods and analysed for total radioactivity, test substance and main metabolites. Strengths and weaknesses of the system for providing supporting information for a detailed risk assessment are discussed.

INTRODUCTION

In order to improve information on the fate and effects of chemical substances in the aquatic environment experimental test systems simulating natural stream ecosystems are of considerable value (Crossland *et al.*, 1991). Small streams are different from static aquatic systems regarding fate and effects of pesticides:
- they experience different exposure patterns as a result of drainage and waste water;
- they are continuously open systems concerning the input and output of substances via waterflow, resulting in downstream transport and dilution following 'spot' contamination;
- the continuously overflowed sediment is exposed to a relatively larger potentially contaminated water volume resulting in greater sorption of lipophilic and persistent chemicals;
- stream biocenoses are dominated by sediment inhabiting species with rather long generation times compared to planktonic species dominating ponds; representatives of important orders with high oxygen demand only survive in stream sediments, e.g. Plecoptera or Turbellaria.

A realistic simulation should be based on an experimental system supplying sufficient size and complexity for self-preservation and consisting of populations of natural age structures. It should enable studies of the fate and effects of chemicals in locations of increasing distance from the treated area as well distinct current velocities and microhabitats. For a detailed risk assessment it is essential to obtain a maximum amount of data about the fate of the pesticide, e.g. by the use of radiolabelled substances in environmentally relevant concentrations. The aim of this project was to obtain initial results about the behaviour of a ^{14}C-pesticide in a new outdoor artificial stream system to evaluate the advantages of such a system for delivering information for a detailed risk assessment in addition to laboratory experiments.

EXPERIMENTAL

Artificial stream system

The artificial stream was installed in the outdoor facilities of the Fraunhofer-Institute, Schmallenberg, and consisted of a stainless steel oval with the main trough 35 m long and 0.6 m wide and with pool and riffle sections included (Debus *et al.*, 1996). The system was filled with about 3 m^3 sediment (10-15 cm depth) and about 2 m^3 water (8-11 cm deep) taken from a natural reference stream within a water protection area (Wenne, Schmallenberg). Stainless steel cages with rainbow trout were installed at sites A-D (Figure 1). The water level was constant during the whole testing period, the water flow was controlled by a paddle system. The velocity of flow varied from 0 to 30 cm/s, the mean value was about 4-5 cm/s. The water temperature decreased from about 10°C at the beginning to 1°C at the end of the study; the water pH of 8.7 was constant over the whole period. The particle size fractions of the sediment at different sampling sites were determined to be: > 2 mm: 45-61 %, 63 µm - 2 mm: 28-35 %, < 63 µm: 7-26 %. The organic carbon content in the sediment ranged from 0.7 to 2.7 %. Previous studies of the biocenosis of the artificial stream and the natural reference stream over a one year period (Debus *et al.*, 1996) revealed that the overwintering populations of the macroinvertebrates showed a comparable development until emergence of the insect larvae. This gives the opportunity to use the natural reference stream as control system for ecotoxicological investigations on benthic macroinvertebrates.

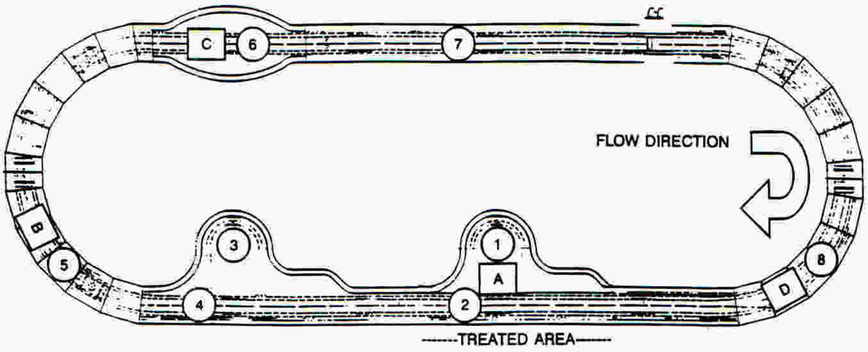

Figure 1. Scheme of the artificial stream system. 1-8: sampling sites; A-D: fish cages.

Treatment

The acaricide amitraz (N-methylbis(2,4-xylyliminomethyl)amine) was used to investigate strengths and weaknesses of the chosen artificial stream mesocosm design, especially whether it is suited for a simulation of spot loading and varying stream currents. Because of the adsorptive properties of the test substance and its fast degradation in water/sediment (Allen & Arnold, 1990) a pure recirculating design was chosen. ^{14}C-Amitraz (specific radioactivity 323 MBq/g) was applied as a 'Mitac^{R} 20EC' formulation in October 1995 at a rate equivalent to the usual field application in hops (2.5 kg a.i./ha). The application solution (product in 600 ml water equivalent to 1500 litres/ha) was sprayed onto the water surface in an area of about 4 m^2 near the first bay (sampling sites 1 and 2; Figure 1). There was no water flow during the application to prevent an immediate equal distribution of the test substance. Two hours after application the normal flow rate was adjusted.

Sampling and analytical methods

Water and sediment samples (9 cm diameter, 15 cm depth) were taken at certain time periods and analysed for total radioactivity, amitraz and the main metabolites BTS 27919 (form-2′,4′-xylidide) and BTS 27271 (N-methyl-N′-(2,4-xylyl)formamidine). Water samples were analysed for total radioactivity by liquid scintillation counting (lsc) and extracted with dichloromethane. Total radioactivity in sediment samples were analysed by combustion. For further analysis the samples were extracted as described by Allen & Arnold (1990). Radioactivity in organic extracts was characterised by lsc and tlc (Merck silica gel 60 F_{254}; cyclohexane/ethyl acetate/triethylamine - 5:3:2 by vol.). Algae samples (periphyton) were blotted dry and the accumulated amount of total radioactive material was determined by combustion. Fish cages were stocked with 30 juvenile rainbow trout (*Oncorhychus mykiss*) each. Samples of 3 fish per cage were taken at each sampling date. They were blotted dry to obtain the fresh weight before drying at 80°C overnight, followed by the analysis for accumulated total radioactivty.

RESULTS

Water

Although there was no waterflow directly after application the applied formulation immediately drifted at the surface in both directions. The highest concentrations of ^{14}C-amitraz 1 h after application occurred in the treated area (sampling sites 1 and 2) and at sampling site 8 (Figure 2). But even at the most distant sampling sites 6 and 7 ^{14}C-amitraz could be detected. In the course of the study the test substance rapidly degraded to BTS 27919, BTS 27271 and non-extractable, polar residues (Figure 2); the DT_{50} of ^{14}C-amitraz was < 1 d, the DT_{90} amounted to 3.5 d. In total 20 % of the initial applied radioactivity were detected in the water phase during the first week, only 5 % remained after a testing period of 50 days.

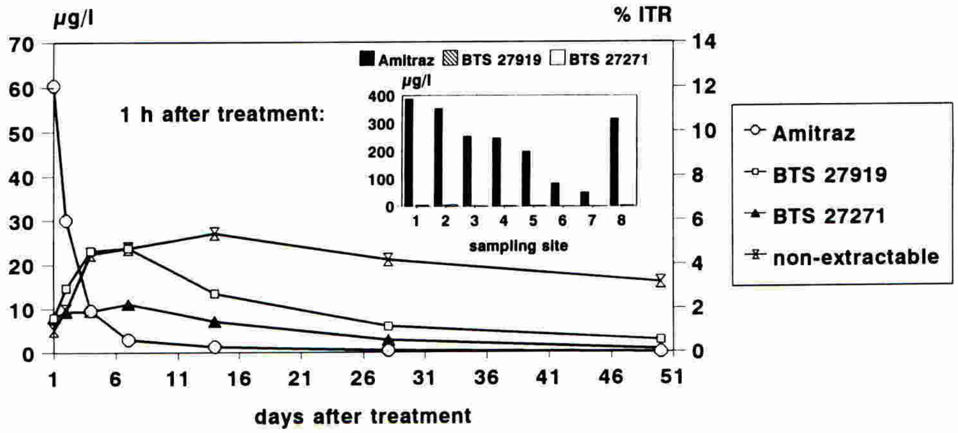

Figure 2. Fate of ^{14}C-amitraz in the water phase. Inset: ^{14}C -amitraz and metabolites in the water 1 h after treatment (no water flow). Non-extractable residues are given in test substance equivalents. % ITR: % of the initial applied total radioactivity.

Sediment

The major part of the radioactive material in the sediment accumulated in the $\leq$ 2 mm particle size fraction of the 0-5 cm layer (Figure 3A). At most of the sampling sites the amount of radioactive material adsorbed to this layer increased during the study. The significant lower concentration of radioactive material at sampling site 3 compared to the other sites was due to the direction and rate of the water flow in this area. Intensive algae growth and/or slack water in front of the fish cages B and D resulted probably in the unexpected high concentration of radioactive material at sampling sites 5 and 8 24 h and 48 h respectively after application. After that the concentration profiles over the remaining study period were comparable to the other sampling sites. In the course of the study radioactive material was transfered into the 5-15 cm layer, increasing to about 30 % of the radioactivity in the upper layer after 50 days. The concentration of total radioactive material in the sediment skeleton (> 2 mm) was generally below 0.1 mg/kg. No ^{14}C-amitraz was detected in the sediment at any time, so its degradation rate was even more faster than in the water phase. The metabolite BTS 27919 was the main degradation product in the 0-5 cm layer. It was present already 1 h after treatment and could still be detected after 50 days (Figure 3B). The rest of the radioactive material corresponded to not identified or non-extractable material, predominant also in the 5-15 cm layer.

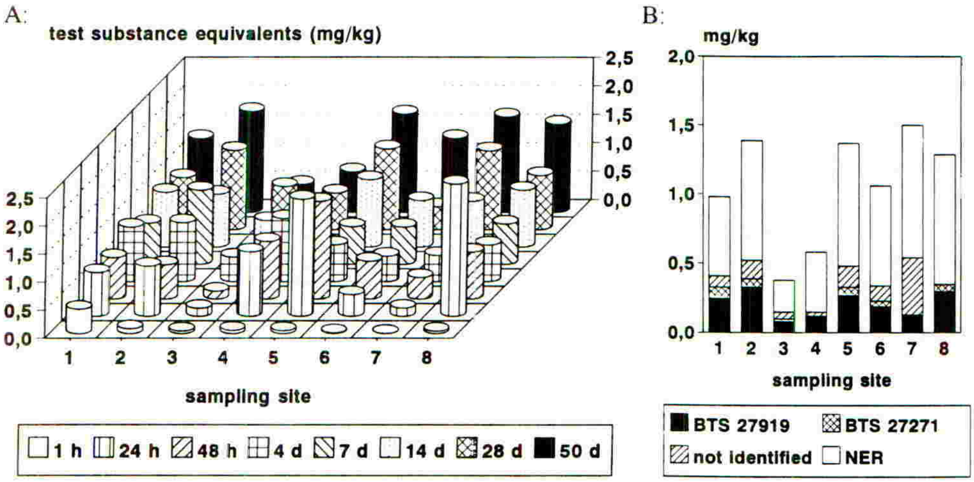

Figure 3. ^{14}C-Residues in the 0-5 cm sediment layer (particle size ≤ 2mm). A: Distribution of total radioactivity over the whole study period. B: Extractable ^{14}C-residues in samples taken 50 days after treatment; NER: non-extractable residues.

Algae (periphyton)

The highest concentrations of total radioactivity in algae occurred up to 24 h after treatment at sampling sites 1, 5 & 8 (0.1 - 0.15 mg test substance equivalents/g). The distribution pattern over the different sampling sites 24 h after application corresponded to the results obtained for the sediment with the unexpected high amount of radioactivity at sampling sites 5 & 8. In the course of the study the concentration of radioactive material in the algae fraction decreased at all sites (< 0.05 mg/g after 48 h) due to elimination of polar degradation products.

Concentration of radioactivity in fish

The amounts of radioactive material accumulated in the exposed rainbow trout 1 h after application were in good agreement to the different initial concentrations of the test substance in the water phase at the various sampling sites (Figure 4). When the normal water flow was established no further differences were observed for the accumulation rates in the distinct areas of the artificial stream.

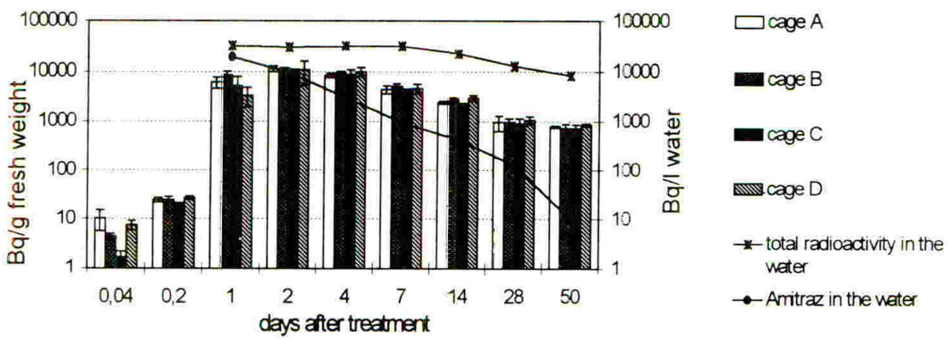

Figure 4. Accumulation of total radioactivity in juvenile rainbow trout, compared to the concentration of total radioactive material and test substance in the water phase.

The maximum concentration of total radioactive material in fish occurred 48 h after application and declined more rapidly than the total radioactivity in the water phase. The latter is the consequence of the fast disappearance of the test substance from the water phase and the formation of more hydrophilic degradation products resulting in a shift of the dominant processes from uptake to elimination. The maximum concentration in fish was approximately 400 times that in water at day 2, representing one third of the steady-state bioconcentration factor determined in a flow-through laboratory test with bluegill sunfish (Allen, personal communication).

CONCLUSIONS OF FATE EXPERIMENTS

The fate of ^{14}C-amitraz in water and sediment of the artificial stream mesocosm was in good agreement to the results obtained by Allen & Arnold (1990) in laboratory experiments. The upper sediment layer contributed most to the rapid degradation of ^{14}C-amitraz in the stream mesocosm. The recirculating design led to a uniform distribution of radioactive material, even in the case of a rapidly degraded adsorptive substance. The great amount of polar metabolites was responsible for the low accumulation and fast elimination in fish. The local exposure of sediment and algae over the total study period was mainly influenced by the relation between water/sediment-equilibrium and current velocity. The high initial concentration in the water phase of the treated area was less important for the long term accumulation in the whole system.

For further studies a more precise generation of diverse current zones and a modification of fish cages are necessary. A simulation of a real spot loading will be performed by removing the contaminated water after nearly one turn into a 5000 l reservoir and simultaneously refilling the system with untreated natural water. This initial once-through-design will enable the simulation of linear transport and natural dilution as well as of an untreated upstream control area.

OUTLOOK: ECOTOXICOLOGICAL STUDIES IN A SINGULAR SYSTEM

Because of size and complexity, only one system for the use of radiolabelled substances was installed in the outdoor facilities of the institute. This raises questions concerning the statistical treatment and the interpretation of results.

Untreated control

Dependent on the generation time of the chosen indicator species and on season, three possibilities of controls with different qualities are available (Table 1). A combination of different controls guarantees the most founded interpretation.

Replicate testing

In the presented artificial stream, statistics can only be based on pseudoreplicate sampling. Thus the results cannot be related to the respective treatment in general, but only to the treated system, just as it would be the case in natural streams. However, some arguments are in favour of this kind of sampling. From complex artificial streams with the same sampling intensity more information can be derived by investigating different microhabitats in one stream than different replicates. The representation of the artificial stream for natural systems is of greater importance than a statistical mean: 17 of 35 large artificial stream studies were conducted without replicates (Lamberti and Steinman, 1993). The macroinvertebrate biocenosis between the emergence periods is very constant compared with a pond plankton biocenosis. Due to the slower population dynamics of the dominant species, a singular stream system provides more predictive certainty than a singular pond system. Previous artificial stream experiments (e.g. Crossland *et al.*, 1991) were conducted under permanent flow-through conditions in several smaller systems. For many of those experiments identical systems were not used as replicates, but for different concentrations: the statistical information about each of the different conditions and even for variance analysis-based statistics were derived from pseudoreplicate sampling.

Table 1. Strengths and weaknesses of controls of different quality.

	strength	weakness
internal control area	part of the treated system best comparability of microhabitats meiobenthon, periphyton short generation times	slight contamination, no zero level no statistical independence macroinvertebrates migration
external control system	best comparability to the entire system statistical independence macroinvertebrates (periphyton) migration	expensive possible divergence of meiobenthon and periphyton biocenosis
natural reference stream	best comparability in terms of reality statistical independence macroinvertebrates migration	input out of management small usable time window between emergence periods rainfall events

Concentration-related effects

In terms of a realistic risk assessment, a main weakness of laboratory studies as well as of most of the previous studies on artificial streams (e.g. Crossland *et al.*, 1991) is the treatment with constant concentrations. Thus, neither dilution after peak loading nor degradation are incorporated in those test designs. Both time and space related decreases of pesticide concentrations can be simulated in the presented design. Effect data can be related to different initial concentrations at different locations in the system and used for ECx statistics. The results have to be interpreted carefully because due to migration it may be that the samples are not independent. As follows, the concentrations related response in terms decreasing abundance is not only the result of lethal and sublethal effects. It is amplified by flight reactions and smoothened by immigration from more contaminated upstream locations. This complicates the comparison to laboratory test data but simulates well the real situation. Nevertheless, the ecotoxicological results cannot be related to the initial concentration in general, but only to the initial concentrations of a downstream succession of locations. Whether this weakness is a severe one has to be discussed on the basis of further data.

ACKNOWLEDGEMENTS

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ESTIMATING THE FATE AND BEHAVIOUR OF PESTICIDES IN SURFACE WATER IN RELATION TO EC-GUIDELINE 91/414/EEC

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ABSTRACT

The FOrum for the Co-ordination of pesticide fate models and their USe (FOCUS) consists of a group of experts from regulatory authorities, industry and research institutes. The main task of the group is to give guidance to the application of mathematical models for the estimation of fate and behaviour of pesticides as mentioned in EC-guideline 91/414/EEC. For surface waters an inventory has been made of applicable mathematical models and their potential for describing loadings to water or for describing pesticide fate and behaviour in the water and sediment phases. A strategy was proposed for the development of scenarios. Finally an example scenario calculation was performed. It could be concluded that the validation of models on a Community level is a major problem. In the near future it is recommended to improve the validation status of the models, to develop an integrated model and to identify relevant scenarios for different European regions.

FOCUS - ORGANIZATION

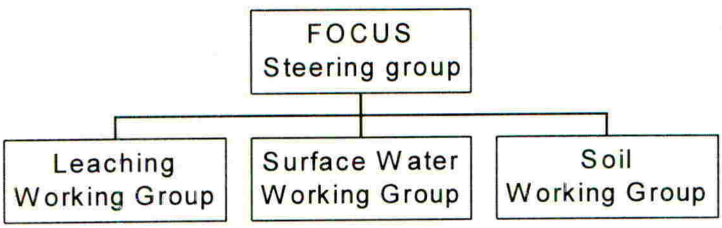
The Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market describes the requirements which have to be fulfilled in order to obtain an authorisation for a plant protection product. The Directive has given great importance to the calculation of Predicted Environmental Concentrations (PEC s) which are then used for conducting further experiments or as a support for evaluation and decision making. It is suggested that PEC s are calculated using a suitable model or calculation method.

Since the regulatory use of simulation models is quite new, there are presently neither clear and detailed guidelines nor a generally agreed practice on how they are to be used and how the results are to be interpreted. The role and importance of models for calculation of PEC s depends strongly on their quality, which can be established through a validation process. Currently no models are validated at a Community level.

The intention of FOCUS (FOrum for the Co-ordination of pesticide fate models and their Use) is to provide industry and regulators with expert advice on the state-of-the-art in simulation modelling and to give the researchers a clear view of deficiencies in the present state of simulation models when used in a regulatory context. FOCUS is a group of regulators, industry representatives and experts from research institutes. The work is co-ordinated by a Steering Committee. The current organisation is depicted in Figure 1.

The Leaching Working Group was the first group established by the Steering Committee and has prepared a document that was released by the European Commission in December 1995 (documents 1694/VI/95 and 4952/VI/95) The Soil Working Group and the Surface Water Working Group have completed their work and draft reports have been presented to the Steering Committee.

Figure 1. Organisation of FOCUS



SURFACE WATER ENTRY ROUTES

The main entrance routes of plant protection products and active ingredients into surface waters were identified as:

- Spray drift
- Surface run-off
- Drainage
- Atmospheric Deposition.

Of course there are other routes possible e.g. incidental releases from cleaning tanks, or accidental releases, but these were considered not to conform to Good Agricultural Practice (GAP) and were thus not considered for inclusion in the modelling process for registration. Atmospheric deposition was not covered by the group to be a major entry route and because work is already carried out on this topic by other fora such as the European and Mediterranean Plant Protection Organisation (EPPO).

DESCRIBING FATE IN WATER

The mathematical description of fate and behaviour of pesticides in the environment is always based on common scientific principles such as conservation of mass. It should be born in mind that a model is a simplification of reality. The simpler the model the greater is the deviation from real observed phenomena. On the other hand, whereas a complex model may simulate many actual processes, it will require a far wider range of input data describing environmental parameters which may be difficult and/or expensive to measure. The models described in this paper are considered to be the most sophisticated models currently available depending on the intended use. Factors determining the usability of surface water models are related to the following items:

- Load. Which discharges into the surface water body are taken into account.

- Mathematics: What mathematical representations are used for the processes taking place, e.g. linear or Freundlich sorption, first order or Michaelis-Menten kinetics for the degradation
- Solutions: Which solutions are possible for the differential equations: analytical or numerical
- Validation: Is the model validated using independent data, and have a sensitivity analysis and a model verification process been carried out
- Sediment: The possibility of calculating the PEC in sediment has been added to the remit of the group and this item has therefore been included in the evaluation process.

THE MODELS

After cataloguing the available models that are currently, or could be used in a regulatory context those presented in Table 1 were selected for a more in depth description.

Table 1. Selected models for the several items of consideration

Drift	Drainage	Run-off	Atmospheric Deposition	Fate in surface water
IDEFICS	CHAIN_2D 1.1	EPIC	none	ABIWAS
MOPED	CRACK_P 1.0	GLEAMS		EXAMS
PEDRIMO	MACRO 3.1	OPUS		SLOOT.BOX
PSMDRIFT	OPUS 1.63	PELMO		TOXSWA
TABLES-NL	PESTLA 3.0	PRZM2		WASP
- UK	PESTRAS 2.1	SWRRBWQ		

The models have been arranged in alphabetical order.

In the final report of the group these models are compared with each other for an extensive list of items, including documentation and systems considerations (user manual; support; input/pre-processor; output/post-processor) and model science (model philosophy; compartments considered; numerical technique). The items may vary depending on the entry route considered.

ADVANTAGES

Each model has its own specific advantages and disadvantages. These include the degree of complexity and the purpose for which it was developed. For example, a model designed principally to simulate a particular process such as surface run-off, may perform better in predicting surface run-off inputs, than another, more general model designed to simulate a range of processes including leaching and drainage, but which simulates surface run-off in a less mechanistic way. This may seem trivial but should always be kept in mind when evaluating models.

The group considered the following criteria to be of major importance when assessing model advantages:

- PEC in sediment; the potency of surface water fate models to estimate a concentration in the sediment phase of the aqueous environment was part of the remit of the group and is part of the data requirements of guideline 91/414/EEC. On the other hand, models not considering the sediment phase estimate the concentration in the aqueous phase and from that estimate a concentration in sediment can be estimated using equilibrium partitioning.
- Ease of use; because the models considered are to be used in a regulatory context - and because in such a context, model users are unlikely to be the model developers or researchers, their user friendliness is important. The availability of standard scenarios for the regions under concern still have to be developed.
- Commonly available; when a model is used in regulatory decision making for the registration of plant protection products in the EU it should be easily available to all potential users.
- Validated; the guideline 91/414/EEC states that if the possibility exists the concentration should be estimated using a suitable model validated at the Community level.

DISADVANTAGES

The disadvantages and/or limitations of the models presented are more or less the opposite of the advantages. More specifically the following items are taken into account:

- Limited to water phase; several surface water fate models do not calculate the concentrations of a pesticide in the sediment phase. Therefore, another method is needed to take care of this calculation. Mostly, equilibrium partitioning between the water and sediment phases is used as an approximation of the distribution. If the sorption capacity to soil and/or sediment is known a reasonable estimation is possible, otherwise the n-octanol/water partitioning coefficient can be used to make an estimation.
- Complex expert use; most of the models have just recently been developed or are still being developed. The experience with the models is therefore generally limited to the researcher or developer of the model. Because of this, most regulatory users, be they industry or authority, are not familiar with the models, their limitations and what may be the most suitable standard scenarios to use.
- Research tool; in relation to the former point it is clear that the models are often used as research tools in the hands of the developer. Thorough testing by the researcher should be normal practice, of course, but once developed, specific research versions need to be adapted, calibrated and validated for a specific regulatory usage. If further model development by the researcher then takes place, this process has to be repeated before the updated model can be used for regulatory purposes.
- Not validated; the current validation status of all the models is considered to be low. Some models are only validated in a very specific situationA lot of work is needed before any model can be considered to be validated at the Community level.

SCENARIOS

In several Member States of the European Union scenarios have been developed to evaluate the behaviour and effects of pesticides in surface waters. They differ in specific assumptions, like e.g. the depth of the water body, etc. Although such an approach is certainly possible, the group recommends the development of European scenarios in relation to a 4 step modelling strategy:

- Step 1: Standard European surface water scenario (based on maximum annual application rate and specific dimensions of a surface water and associated sediment)
- Step 2: Extended standard European surface water scenario (based on a sequence of applications and an estimation of rates of loss from a standard surface water and sediment scenario)
- Step 3: Worst case scenario including all inputs (spray drift, runoff and drainage) and a standard surface water and sediment scenario.
- Step 4: Specific scenario simulation taking into account specific combinations of cropping, soil, weather, field topography and aquatic bodies adjacent to fields.

Scenarios developed by member states can still play an important role in the registration of plant protection products. Each country needs to evaluate specific products for which registration in that country is sought, although the active ingredient may have been included on List 1 already. In this case an idea about the concentration levels to be expected in the national scenario is useful for reference to the local administrators.

Looking at the map of Europe it is quite clear that there will be a wide variety in climatological and hydrological conditions from northern Sweden and Finland to southern Spain, Italy and Greece. Of course, it is not possible to fit this variety into a simple scenario, but several characteristics of the regional difference can be built into a number of scenarios to be developed.

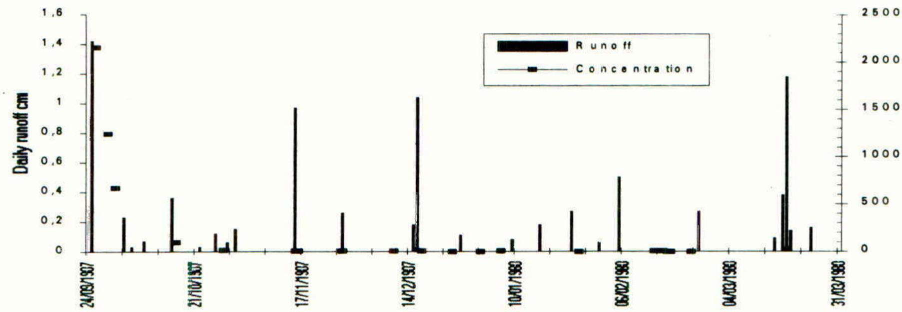
In the strategy mentioned above worst case assumptions should be applied in step 3. A time series of surface water loadings relating to 'worst case inputs' via spray drift, drainage, or surface runoff is required. It is however recommended to use a limited set of 'worst case' assumptions to avoid unrealistic situations. In each case, the definition of worst case conditions will depend on the critical parameters used in the chosen model.

EXAMPLE CALCULATION

An example scenario calculation was carried out using one of the models of each group mentioned. The example was based on a hypothetical herbicide (soil half-life = 50 days, Koc = 100) applied early post-emergence in autumn to a drained, clay soil. The simulation used a climate scenario where 550mm of rain fell during the period October to April with 80mm in the first 30 days post-application. Results from this example are presented in Figure 2. The models were:

- drift: the German drift table (Ganzelmeier *et al.*, 1995)(average of 2.5% drift following an application 1m distant from a 2m wide ditch)
- run-off: GLEAMS (dimensions of field 100m x 100m, 2% slope towards ditch)
- drainage: MACRO (drain spacing 10m intervals; drain depth 0.8 m from the surface)

a)



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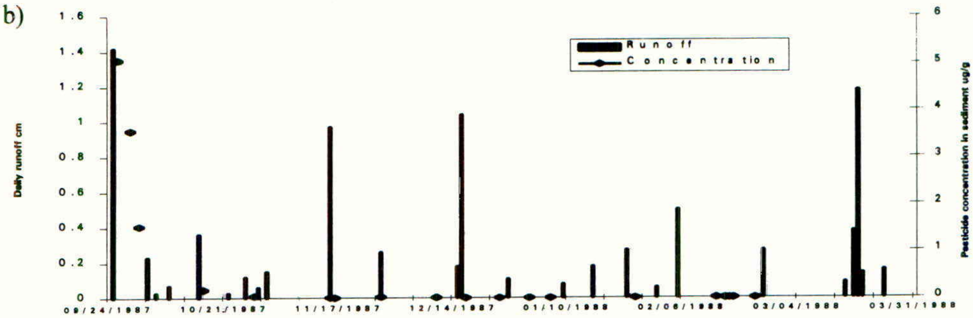

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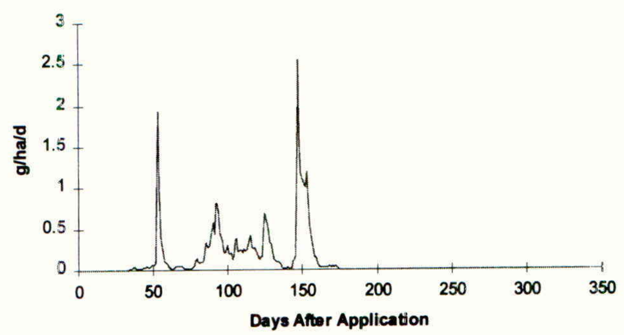

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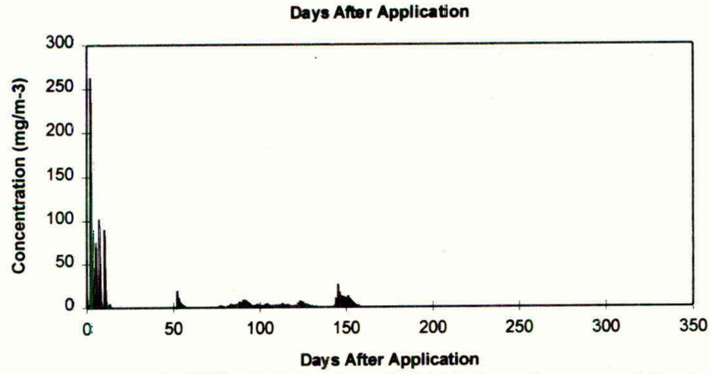

Figure 2. Results of an example PEC calculation for a herbicide in surface water following application in autumn to an adjacent clay soil field. a) runoff inputs in the liquid phase, b) runoff inputs in the solid phase (both calculated with GLEAMS) c) subsurface mass transport via drains (MACRO) and d) resultant surface water concentrations calculated with EXAMS (including the input from spray drift on day 0. See text for more details.

- fate: EXAMS (ditch 2m wide x 100m long running the length of one side of the field, water 50cm deep, flow rate 0.001m/s)

It should be stressed, however, that use of the models here in the example calculation does not mean that they can be considered as the preferred models. The results of the group's evaluation of models still stands.

CONCLUSIONS AND RECOMMENDATIONS

The main conclusions of the FOCUS Surface water Modelling Group are:

There are currently no at the European level validated models available. Several models are locally validated or being validated to a limited extent. Other models are not validated at all and because they are not further developed will not be validated in future. For some models the validation status is rising. Validation studies are in progress already or have been scheduled in the near future. These models must be considered as the most promising ones.

There are currently several useful models available for simulating surface water loadings via the various entry routes defined by the group. For spray drift, the best results are obtained using drift tables, e.g. the German drift table combined with the simple interpolation model PSMDRIFT. With respect to drainage the most useful models are considered to be PESTLA, CRACK_P and MACRO. Concerning runoff, the models GLEAMS, PELMO and PRZM are considered to be most applicable. And finally, for fate the models EXAMS, TOXSWA and WASP are considered to give the most useful results in estimating the concentrations of pesticides in surface waters and sediment.

During the evaluation of surface water behaviour of pesticides a tiered approach must be considered most promising, because the most detailed and complicated modelling is only required when absolutely necessary. In particular, the screening models ABIWAS and SLOOT.BOX may give useful results first or second step results in combination with a defined standard European scenario.

As the European evaluation of pesticides is just starting it is not surprising that standard European scenarios are lacking. However this is considered to be a serious problem for the development of a harmonised European approach to estimating PEC s.

Finally, the group comes to the following main recommendations:

Research should be carried out for drift data in Southern Europe. All of the drift data come from countries in the west or north of the European Union. It is questionable if these data can be extrapolated to southern Europe. However, efforts should be made to extrapolate and validate the current models for southern European conditions.

Validation of all models considered is urgently needed, especially in view of the wording in the EU guideline 91/414/EEC. If validation at community level is not yet possible, models should only be used for the situation they are validated. In particular, validation efforts should be focused on the following:

- Runoff curve numbers, as they are only empirically established for US situations,
- Drainage, especially on the community level,
- Fate in surface waters, as work in this area has been started only recently (e.g. TOXSWA).

Development of European scenarios. Registration of active ingredients has to be approved by the Commission of the EU taking into account European circumstances. Only the registration of specific products belongs to the competence of the local designated national authority. This common registration procedure can not function without the availability of suitable scenarios within the European Union.

Model development. There is no model available describing all the input routes and behavioural aspects of plant protection products in the European Union. Such a model could be constructed building on elements of the available models for the different input routes and the fate models themselves. As has been shown in the example calculation using output of one model as input to the next model is possible but is not considered easy. It is time consuming and expensive. Streamlining this process is strongly recommended.

Interpretation of model results. An independent problem arising from using models is the interpretation of the model results, certainly in the light of the consequences for the registration or refusal of a registration in the EU. Model developers, model users and decision makers should work together in gaining knowledge on how to interpret the results and if necessary to carefully balance an appraisal.

ACKNOWLEDGEMENTS

It would have been impossible to prepare this paper without the input of the members of the FOCUS working group on Surface Water Modelling. Therefore, I would like to express my sincere thanks to first of all John Hollis (SSLRC, UK), secretary of the group, who did an excellent job combining it with his busy normal work load and to the other members: Pauline Adriaanse (SC-DLO, NL), Richard Allen (AgrEvo, UK), Veronique Gouy (Cemagref, F), Jürg Hosang (Ciba Geigy, CH), Nick Jarvis (SLU, S), Tim Jarvis (PSD, UK), Michael Klein (IUCT, G), Raymond Layton (Dupont, USA), Helmuth Schäfer (Bayer, D), Louis Smeets (European Commission, B) and Denis Yon (DowElanco, UK).

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DRIFT REDUCTION IN CROP PROTECTION: EVALUATION OF TECHNICAL MEASURES USING A DRIFT MODEL

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ABSTRACT

To describe downwind spray deposits from a conventional boom sprayer in arable farming the IDEFICS drift model was developed. The model takes account of many important factors involved with the spraying process.
Different technical measures to reduce spray drift outside the treated field are evaluated using the model. These measures include (a) the use of low-drift and (b) coarse spray nozzles, (c) an asymmetric 'edge nozzle' at the tip of the boom, (d) reducing boom height above the crop, and (e) allowing an unsprayed zone at the edge of the field, the crop still present in the zone. These measures are evaluated both at low and high wind velocity.
Model computations show that the amount of downwind deposits of spray drops can be reduced considerably by technical measures. The relative drift reduction of measures (a), (b) and (d) increases with downwind distance, whereas with measures (c) and (e) relative reduction is highest just outside the field. Combinations of measures may result in high drift reductions at all distances.

INTRODUCTION

Chemical crop protection becomes more and more a political issue (e.g. regarding the pollution of surface water). Therefore drift reduction has gained much attention. However, measures to reduce drift must not lead to decrease of agricultural yield. Several technical measures may reduce downwind spray drift from conventional field sprayers. Unfortunately, direct measurement of spray drift to assess such measures quantitatively is cumbersome. This study deals with the quantification of drift reducing measures using a simulation model to compute downwind spray deposits.

MODEL CONCEPTS

The drift model IDEFICS is a random-walk model to describe downwind spray drift from a conventional boom sprayer in a cross wind (see Figure 1). Trajectories and downwind deposits of spray drops produced by a flat fan nozzle are computed. Downwind, trajectories are restrained by an imaginary vertical boundary. The model basically is two-dimensional (2D), although close to the nozzle driving speed is accounted for, introducing a third dimension. Trajectories are affected by crop related parameters (e.g. crop height), application related parameters (nozzle type and size, liquid pressure, boom height, driving speed), and atmospheric parameters (wind velocity, turbulence, relative humidity, temperature).

Size spectrum, angular distribution and initial velocity of drops - input data for the model - were

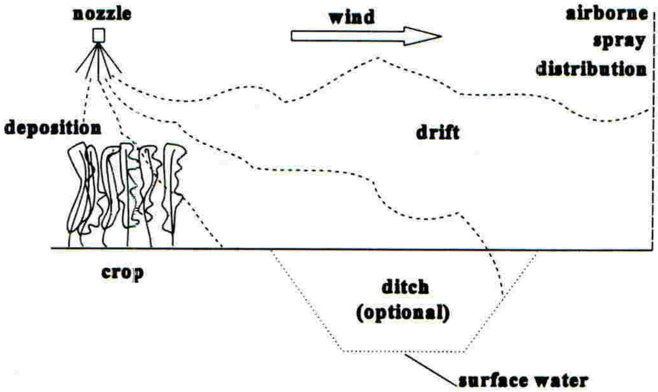

Figure 1. Schematic field layout for the simulation model.

measured using phase-doppler anemometry. The model accounts for entrained air movement below the nozzle, a logarithmic wind profile, atmospheric stability, and evaporation of drops. Spray drift of a whole sprayer boom is calculated by summing the drift computed separately for a representative set of nozzles along the boom.

A comprehensive description of the IDEFICS model including validation is given by Holterman *et al*. (1996).

DRIFT REDUCING MEASURES

Technical measures to reduce spray drift can be classified into three groups, containing measures that

(i) reduce the amount of drift-prone drops (e.g. using coarser or so-called low-drift nozzles);
(ii) control the direction of drops towards the target (e.g. air-assistance, shielding of the sprayer boom, asymmetric 'edge nozzles' at the boom tip to prevent spraying directly over the edge of the crop, lowering the sprayer boom);
(iii) increase the distance between spray source and area outside the field (e.g. unsprayed boundary zone around the crop field, with or without a crop present).

Strictly speaking, an unsprayed boundary zone does not reduce drift at all. It merely reduces its symptoms to the area outside the crop field. A drift reducing measure somehow limits the amount of drops drifting a certain distance downwind. However, its effectiveness may differ at different downwind distances. In general a combination of technical measures will give a better drift reduction than a single measure, although the overall drift reduction is less than the sum of the individual reduction percentages.

COMPUTATIONS

To quantitatively investigate technical measures to reduce drift, several spraying applications were compared with a chosen reference situation. The reference situation was a conventional application to a crop of 0.5 m height in a cross wind, with the sprayer boom at 0.5 m above the crop, and medium-sized flat fan nozzles (XR11004 @ 300 kPa). Driving speed was set to 1.5 m/s.

The following measures to reduce spray drift were selected for computations:
(i) using 'low-drift' nozzles (DG11004 @ 300 kPa);
(ii) using coarse nozzles (XR11008 @ 300 kPa);
(iii) using an 'edge nozzle' (off-centre nozzle OC04 @ 300 kPa);
(iv) decreasing boom height above the crop down to 0.3 m;
(v) allowing an unsprayed boundary zone (1.0 m width; crop present);
(vi) combinations of measures (i+iv, i+iv+v, iii+v, i+iii+iv, i+iii+iv+v).
Their effect on spray deposits was computed as a function of downwind distance from the edge of the crop, at both low and high wind velocity (2 and 5 m/s respectively). Although obtaining a high drift reduction percentage at each distance is best, one must realize that the bulk of drifting drops will deposit the first few metres downwind.

Results

Drift reduction as a function of downwind distance is shown in Figure 2 for single technical measures at an average wind velocity of 2 and 5 m/s. Curves (a) and (b) show that using coarser nozzles relative drift reduction was constant beyond 2 to 3 m downward.
Using an asymmetric 'edge nozzle' at the boom tip resulted in a high reduction percentage just outside the sprayed field. However, curves (c) go below zero from about 3 m downward, which in fact corresponds to increased spray deposits. This was probably due to drops from the upper part of the spray cone, which started almost horizontally and directed upwind. Due to their lack of downward initial velocity they were easily blown away in a cross wind.

Lowering boom height from 0.5 m down to 0.3 m above the canopy reduced downwind deposits considerably (curves (d)).

Even allowing only 1 m unsprayed at the field edge had a considerable effect on downwind deposits (curves (e)). In this case the crop was still present in the unsprayed boundary zone.

It should be noted that an 'edge nozzle' and an unsprayed zone were effective just outside the sprayed field, while using coarser nozzles or lowering the sprayer boom were more effective beyond 2 to 3 m downwind. An apt combination of measures may therefore be complementary. Figure 3 shows several combinations of drift reducing measures at an average wind velocity of 2 and 5 m/s. Most combinations resulted in an high average drift reduction percentage, typically around 80%. Using low-drift nozzles together with a reduced boom height still lacked effectiveness nearby (curves (a)). Using an 'edge nozzle' together with an unsprayed zone of 1 m had only poor effectiveness beyond 3 m (curves (c)). Curves (b), (d) and (e) correspond to combinations of measures revealing both short-distance and long-distance effectiveness, resulting in high drift reduction at each distance. From these examples the best combination comprised the use of low-drift nozzles, a reduced boom height and an unsprayed zone (curves (b)).

Table 1 gives the overall drift reductions averaged over deposits 0 to 20 m downwind. While the effectiveness of using an 'edge nozzle' seemed disappointing previously (curves (c) in Figure 2), the overall reduction was better, particularly at low wind velocity. Using low-drift or coarse nozzles was less effective when the overall reduction was considered. Generally, measures with short-distance effectiveness performed better in overall drift reduction, since such measures reduced the total amount of drifting pesticides more than those with long-distance effectiveness.

As noted before, combinations including both measures with short-distance and long-distance effectivity may gain high drift reduction. Very high overall reduction percentages (>90%) were obtained with combinations that included both a reduced boom height and an unsprayed boundary zone.

Although the effect of wind velocity on the absolute amount of downwind spray deposits is obvious, both the reduction curves and the overall percentages show that drift reducing measures responded similarly in low and high wind velocity.

Table 1. Overall drift reduction percentage for various technical measures, averaged over deposits 0-20 m downwind.

measure	overall drift reduction (0-20 m) [%]	
	@ wind 2 m/s	@ wind 5 m/s
low-drift nozzles (1)	6	9
coarse nozzles (2)	12	26
'edge nozzle' (3)	57	32
reduced boom height (4)	23	34
unsprayed zone (5)	89	71
1 + 4	24	39
1 + 4 + 5	98	93
3 + 5	91	70
1 + 3 + 4	87	75
1 + 3 + 4 + 5	98	90

CONCLUSION

Simulation of downwind spray drift can efficiently quantify drift reduction for various technical measures. Relative drift reduction appears to be a function of downwind distance from the edge of the crop. Considering their performance in relation with distance, measures can have short-distance or long-distance effectiveness. High drift reduction, essentially independent of distance, can be obtained using a combination of measures involving both short-distance and long-distance effectiveness. In the Netherlands crop fields are usually surrounded by ditches. Preventing contamination of surface water therefore implies that reducing spray deposits in the range 1 to 4 m from the edge of the crop field has high priority.

Applying technical measures may have some practical consequences. For example reducing boom height above the crop demands high-stability sprayer booms. A measure such as the introduction of an unsprayed zone may lead to yield loss and therefore is likely to be unpopular among farmers. The use of coarse nozzles may lead to decreased crop protection and disease control.

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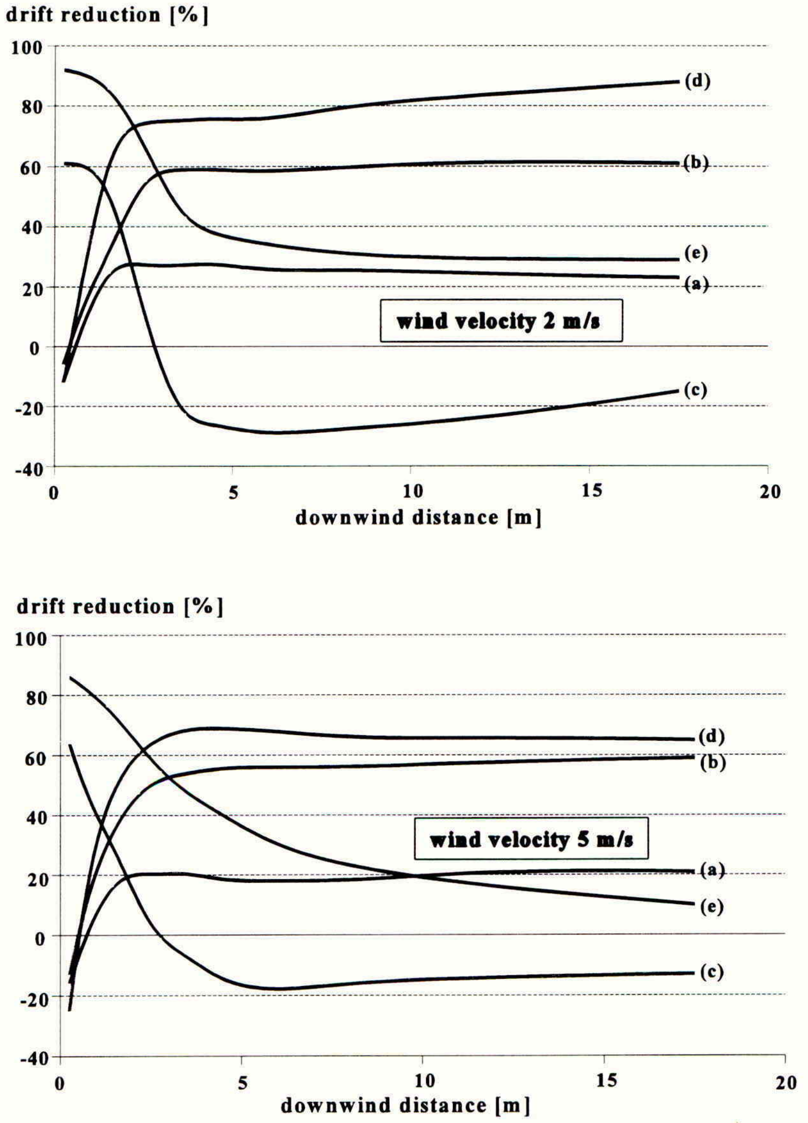
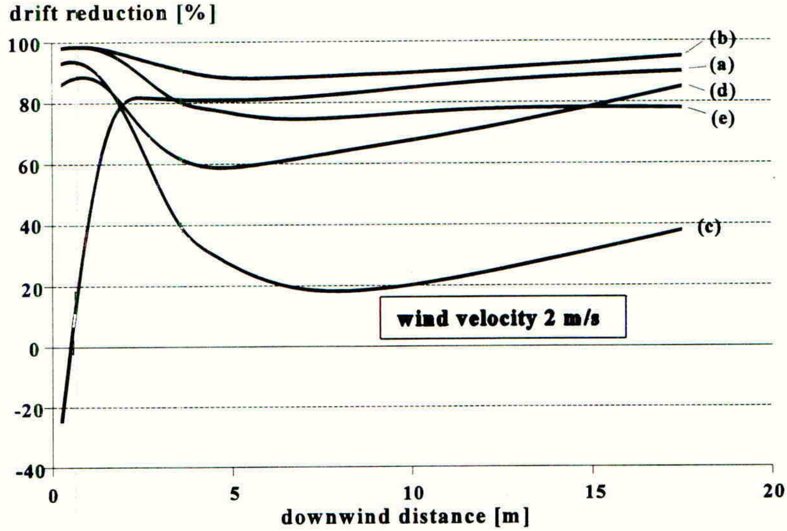


Figure 2. Downwind drift reduction at wind velocity 2 and 5 m/s for various single measures, as a function of distance from the edge of the crop. Measures: (a) low-drift nozzles; (b) coarse nozzles; (c) edge nozzle at boom tip; (d) reduced boom height; (e) unsprayed zone (crop present in zone). Drift reduction percentage is relative to a conventional application to a crop using flat fan nozzles (see text for details).



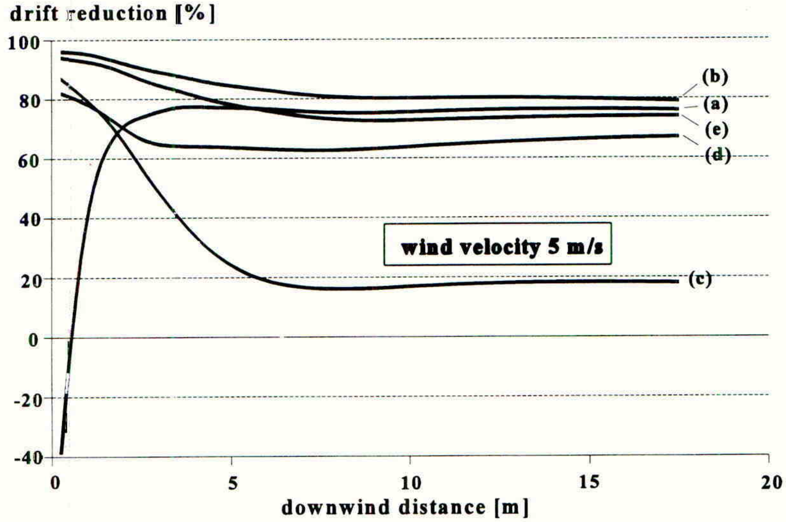


Figure 3. Downwind drift reduction at wind velocity 2 and 5 m/s for various sets of measures, as a function of distance from the edge of the crop. Measures: (a) low-drift nozzles + reduced boom height; (b) low-drift nozzles + reduced boom height + unsprayed zone; (c) edge nozzle + unsprayed zone; (d) low-drift nozzles + edge nozzle + reduced boom height; (e) low-drift nozzles + edge nozzle + reduced boom height + unsprayed zone. Drift reduction percentage is relative to a conventional application to a crop using flat fan nozzles (see text for details).

SESSION 3B

PESTS AND DISEASES IN VITICULTURE – CURRENT PROBLEMS AND SOLUTIONS

Chairman	Dr A P Legge *MACK Organisation, Tunbridge Wells*
Session Organiser	Dr J N Knight *Willmot Pertwee Horticulture, Tonbridge*
Papers	3B-1 to 3B-4

INTEGRATED MANAGEMENT OF GRAPEVINE DISEASES AND PESTS

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ABSTRACT

The aim of integrated management in viticulture is to produce grapes and wine of high quality taking into consideration ecological and economical aspects. To control fungal diseases prognosis models are available or under investigation. Insects and mites are managed by biotechnical methods and antagonists respectively. In practice, all viticultural measures are integrated to control diseases and pests.

INTRODUCTION

Integrated management of diseases and pests in grapes began at the end of the last century with the control of Phylloxera (*Daktulosphaira vitifoliae*) by means of grafting onto tolerant rootstocks. At the same time downy mildew (*Plasmopara viticola*) has been imported from North America into Europe by infected grapevine species used for breeding of appropriate rootstock varieties. Due to the severe losses in European vineyards all attempts had been made to inquire into the causal agent of this disease and its life cycle. In the first decade of this century the relationship between climatic condition and the epidemic of downy mildew was elucidated. Subsequently the first approach to an integrated control by an incubation calendar was made, treating the vines with copper by time and saving needless treatments (Müller, 1912) .

Today in all vine growing regions worldwide efforts are made to establish and promote integrated management of grapevine diseases and pests. There are two aims for this purpose. First of all the economical aspects. The universal objective is to produce grapes and wine of high quality as econimically as possible. Control of diseases and pests is most effective and economical when all factors concerning the vine and its cultivars, the pathogens, insects and mites, the epidemic and dynamic of population, the climatic and enviromental conditions, the cost of treatments including personnel, the viticultural techniques etc. are considered. When all epidemiological and viticultural factors are integrated the treatments can be focused and their efficacy and economical implication will be raised. The second aspect is the ecological impact of fungicides, insecticides and acarcides. Integrated control results in a substantial reduction both in the number of applications and in the amount of chemical compounds released in the ecosystem.

In integrated management systems all viticultural measures should be achieved in order to avoid inappropriated treatments of pesticides. In the vineyards a high diversity with a high degree of ecological stability should be attained (Boller et al., 1990). For this reason a green cover of the vineyard is essential. Intergrated management demands that the biology and epidemiology including the favourable climatic conditions of the diseases and pests be sufficiently understood. Therefore a substantial amount of basic and applied research is needed to develop appropriated systems. The viticulturists involved in integrated management very seldom have all available informations in a useful form at their disposal when control decisions must be made. For this reason an advisory service must be established to provide sufficient information.

MAIN DISEASES OF GRAPEVINE

Downy mildew (*Plasmopara viticola*)

The cultivars of the European grape (*Vitis vinifera*) are highly susceptible to downy mildew (*Plasmopara viticola*). Under humid conditions the disease spread very rapidly and causes severe losses of yield and quality. The fungus overwinters as oospores in infested leaves. In the spring when temperature increase the epidemic starts during rainy periods. Subsequent to the primary infection sporangia develop and the secondary cycle begins. Continued spread of downy mildew during the growing season depend on the frequency of suitable conditions for sporangia development, sporulation and infection. The period from infection to new sporangia development, the incubation period, depends on temperature and humidity. Infections take place during longer periods of leaf wetness (Blaeser, 1978, Gehmann 1987).

Powdery mildew (*Uncinula necator*)

The causal agent of powdery mildew, the fungus *Uncinula necator*, ore and more spreads due to favourable conditions for its spread in the last 6 years. Powdery mildew affects leaves, shoots, inflorecences and berries. Berry infections cause a loss of quality of the wine because the mycelium influences the taste of wine. In Europe the fungus survives the winter as mycelium in dormant buds. In the spring mycelium grows over the arising shoot tips and young leaves and the shoot tip becomes infected. These „flag shoots“, covered with white powdery mycelium and conidiophores, provide the primary inoculum for the summer cycle. The progress of the epidemic depends closely on the first occurence of „flag shoots“ in the spring. During a warm and dry spring the epidemic starts in a early phenological stage of the vine and cause seriouse infections mainly on inflorescences and young berries.(Fessler & Kassemeyer, 1995, Huber & Bleyer 1996). In North America and southern vine growing areas powdery mildew overwinters also as ascospores in cleistothecia (Pearson & Gadoury 1987).

Bunch rot of grapes (*Botrytis cinerea*)

In a humid late summer and autumn bunch rot (*Botrytis cinerea*) may affect seriously yield and quality of wine. Especially on red cultivars the economical impact is severe because the fungus is destroying the red color in the berry skin by its enzymatic activity. In contrast to the heavy losses sometimes *B. cinerea* is responsible for noble rot when infections take place late in the maturity of berries under certain weather conditions. Moist conditions during the bloom cause infections of the stigma of the flowers. The flower infections become quiescent in the young berries up to the stage of veraison. In the ripening berry the fungus resumes its development due to the influx of sugar. The fungus sporulates on the infected berries and the spores spread by air. The spores germinate in free moisture on berry surface and cause new infections within two days.

MAIN PESTS OF GRAPEVINE

Berry moth (*Lobesia botrana, Eupoecilia ambiguella*)

In European vineyards the two species of berry moth (*Lobesia botrana* and *Eupoecilia ambiguella*) occur. Both species overwinter as pupa on the trunk. In spring, adults of the first generation appear. After mating females lay eggs on grape clusters prior to bloom, in May. Within 8 to 12 days larvae hatch and begin to feed on flower buds and flowers. In July the second generation emerges. In this generation eggs were layed onto young berries. Larvae feed on developing berries boring a small hole in the berry skin. They often move among many berries and invade them causing damage. Additional the affected berries become susceptible to bunch rot.

Spider mite (*Panonychus ulmi, Tetranychus urticae*)

The two species of spider mites (*Panonychus ulmi* and *Tetranychus urticae*) are common in vineyards and are promoted by warm weather conditions. Feeding on leaves by both species causes bronze discoloration and, due to the loss of productive leaf surface, a reduction of quality of the berries. Whereas *P. ulmi* already attack the young leaves after bud burst *T. urticae* migrates onto the vines in summer.

INTERGRATED CONTROL OF FUNGAL DIESEASES

Prognosis models for downy mildew

The findings on biology and epidemiology of downy mildew obtained in the last two decades have permitted the development of prognosis models for applications in confirmity with the actual life cycle. Effective control of downy mildew by fungicide treatments is restricted to a short period from sporulation of the fungus up to the first parasitic stage of the mycelium immediately after the infection. Fungicide applications according the epidemic situation and the life cycle respectively can be timed by means of prognosis models. There are two approaches for modeling downy mildew regarding the acute weather data: (i) the development of fungus and potential infection periods were determined (Kassemeyer, 1994), (ii) the potential infection level were computed (Blaise & Gessler, 1990). Prognosis models based on the climatic conditions favouring the disease cycle of downy mildew have been placed in viticultural practice worldwide. On the basis of temperature, relative humidity and wetness of leaves or berries it is possible to determine the time of sporulation, the viability of sporangia, the time of potential infection and the duration of the incubation period. In order to register and store the weather data and calculate the epidemiological parameters mentioned above, electronic weather stations are available. Strategies for control of downy mildew are carried out according to the information about the epidemic stage of the fungus for which control measures are required as computed by these stations (Bleyer & Huber 1995).

Strategies to control powdery mildew

The aim of integrated control of powdery mildew is to prevent the progress of the infestation in an early epidemical stage. Therefore a strategy for applications were compiled based on the course of the epidemic. According to the findings of 4 year field trials the spread out from „flag shoots" as source of primary inoculum starts when 3 to 6 leaves were well developed on shoots. Moreover there is a close relationship between the number of „flag shoots" and the severity of infestation. Therefore control of powdery mildew is achieved by applying an effective fungicide immediately before the first infections take place. This strategy requires a application with sulfur in the 3-leaf stage. In this stage sulfur is effective, has a side effect on harmful mites (spider mites, grape rust mites and erineum mites) and is harmless to overwintered adult predatory mites. Applications with sulfur should be continued up to the beginning of flowering. Regarding the high susceptibility of flower clusters just before and during blossoming, treatment with DMI-fungicides are recommended in these stages. The strategy to control powdery mildew according to both the development stages of the fungus and the phenological stages of the vine is an approach to integrated management (Huber & Bleyer 1996). Weather driven models which are under investigation will in future provide more effective control of powdery mildew.

Intergrated control of bunch rot

Integration of viticultural methods are most effective measures for the control of grapevine bunch rot. The fungus requires a long period of moisture on the berry surface for infection and spread. Canopy management provides conditions for proper aeration and faster drying of clusters and berries after dew and rain. For integrated control of bunch rot canopy management is absolutely necessary. In the same manner clones with loose clusters have to be choosen due to the reduction of susceptibility for infections. Excessive growth of berries due to imbalance of nutrients promotes rapid development and sporulation of the fungus. Therefore nitrogen fertilization should be reduced to an optimal level. Frequently the fungus invades berries through feeding-sites of grape berry moth. Control of berry moth decreases infestation by bunch rot substantially. Rainfall during blossoming favours flower infection. Additionally under wet conditions in the early stages of berry development after fruit set, dying floral parts and aborted berries trapped in the cluster may become colonized. In this case, two treatments with fungicides are recomanded. The applications should be achieved at the late flowering stage (flower cluster showing 80 % calyptras off) and in the stage before clusters close.

INTEGRATED CONTROL OF PESTS

Control of berry moth by means of mating disruption

Mating disruption by means of specific pheromones has become a more important biotechnical method for integrated control of both species of grapevine berry moth. To obtain an effective control 500 pheromone dispensers per ha were applied in the vineyards. A minimum of 4 ha of treated vineyards is recommended to obtain an effective control of berry moth. The dispensers have to be placed in vineyards before the mating take place. In order to time their introduction, monitoring of flight activity by pheromone traps is required. If the flight activity reaches a maximum the dispensers should be suspended in the canopy.

Control of spider mites

The promotion of predatory mites, especially *Typhlodromus pyri*, is the most important method to control spider mites in viticulture. The occurrence of a sufficient population of adults of this predator in spring may reduce the number of harmful mites to below the critical threshold. Predatory mites were promoted by withdrawal of insecticides and by treatments with fungicides without side effects on *T. pyri*. The predatory mite needs pollen for nutrition of the summer generation. Therefore a green cover with a high diversity of flowering plant species is essential for maintaining a population of *T. pyri*. In vineyards without a sufficient population, green shoots or canes from an other site with a high density of individuals can be suspended in the canopy to colonize the predator.

PERSPECTIVES

At present integrated control of fungal diseases requires the application of fungicides. In viticulture no significant effect of antagonists on parasitic fungi has been found in the field up to now. However the efforts to look for hyperparasitic fungi suitable for integrated disease control in grapevine should be continued. A further approach for integrated control is the induced resistence. Recently biochemical and molecular biological studies on disease resistance response capabilities of grapevine had been made (Busam et al. 1996). The findings suggest that grapevine is capable of responding by systemic acquired resistance using natural and synthetic inductors. In this field all efforts will be made to elaborate a suitable system for integrated control by means of systemic induced resistance. Moreover virgorous plants have reduced suscepibility for fungal diseases. The production of healthy plant material free from graft-transmissible diseases like viruses, bacterias and phytoplasma is an important prerequisite for integrated management of grapevine. Studies on graft-transmissible infectious diseases and their agents were conducted to improve the sanitary status of grapevine propagation material.

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KEY GRAPEVINE PESTS AND DISEASES IN NORTH AMERICA AND RESISTANCE THROUGH GENETIC ENGINEERING

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ABSTRACT

The production of transgenic grapevine will potentially provide viticulturists with many more rootstock and scion options than have been currently available because of pest and disease concerns, as well as offer alternatives to chemical control of grapevine pathogens. The GNA gene has been shown to provide resistance against nematodes as well as Homopteran (sap sucking) insects. Our goal for this program is to achieve increased resistance to these pests in several grapevine cultivars, by genetic transformation with the GNA gene. There are currently many major pests and diseases of grapevine in North America, many of which may be potentially controlled by the GNA. Somatic embryos derived from clonal leaf material were transformed using *Agrobacterium*-mediated transformation. GUS Assay, protein quantification by Western Blot, and DNA analysis by Southern blot were used to confirm transgenic clones. Transgenic clones representing over 100 individual transformation events are currently in bioassay and greenhouse studies. Transformation programs using other gene constructs and other genes of interest are underway in order to provide resistance to a wider range of major grapevine pests and diseases.

INTRODUCTION

Grape production in North America is in excess of 6 million tons annually, with over 5 million tons produced in California alone. This industry losses hundreds of millions of dollars annually due to pest and disease damage and control. Thousands of acres of vineyards are being planted, and replanted annually. There are a plethora of rootstock and scion combinations available to the grower, each specific for individual soil and climate conditions. Often times however, the grower is restricted in the selection of the ideal compound vine because of potential pest and disease problems. A brief synopsis of some of the major pests and diseases facing vintners in North America are listed below.

Bunch Rot

Bunch rot is most commonly caused by the fungal pathogen *Botrytis cinereia.* Botrytis produces enzymes which break down the cutin in the epidermal tissue of the grape berry which can destroy the integrity of the berry in a very short period of time. In addition to the economically significant primary injury, Botrytis allows secondary pathogens access for invasion and further damage. Several chemical fungicides are still available for Botrytis control.

Powdery Mildew (*Uncinula necator*)

Powdery mildew is the most expensive and widespread disease in *Vitis vinifera* vineyards in north America. The fungus infects any succulent epidermal tissue. Infection of the fruit causes blemishes, stunted berries, and off-flavors. Powdery mildew may affect photosynthethesis and sugar production when leaves are infected. Severity of infection depends largely on the grape variety and the regional climates. Tools for preventative management of the disease are available, however repeated sprays, dustings, or leaf removal, can become very expensive.

Pierces Disease

Pierces Disease is caused by the bacterium *Xylella fastidiosa* which is vectored by specific kinds of sharpshooter leafhoppers: The green sharpshooter, *Draeculacephala minerva*, the red-headed sharpshooter, *Carneocephala fulgida*, and the Blue-green sharpshooter, *Graphocephala atropnctata*. The bacterium cause blockages in the xylem of infected leaves, the leaves subsequently yellow and die. In serious cases, infected vines are removed and new vines are replanted. Sensitivity to the disease is cultivar dependent, and management of the disease relies on insecticide control.

Eutypa Dieback

Eutypa Dieback is a canker disease that is caused by the fungal pathogen *Eutypa lata*. Spores of the fungus are dispersed by the rain onto fresh wounds where infection occurs. Disease management consists of wound protection combined with good viticulture practice.

Leafroll Virus

Leafroll disease can cause reduced grape yield and quality. The only control measure is to propagate and plant clean material.

Fanleaf Virus

The fanleaf virus is vectored by the dagger nematode (*Xiphinema index*). Infected vines may be stunted, and have reduced fruit set. The presence of the virus can cause variable berry size. Management of the virus consists of planting clean material, and nematode control.

Grape leaffolder (*Desmia funeralis*)

This Lepidopteran insect in it larval stage feeds on grapevine leaves and sometimes fruit. Yield loss may occur during heavy infestations, due to reduced leaf surface area caused by leaf rolling and feeding of the insect larva. Chemical as well as natural parasites are available for the control of grape leafroller.

Western Grapeleaf Skeletonizer (*Harrisina brillians*)

The larva of this Lepidopteran insect can skeletonize entire grape leaves, leaving only the veins. The larvae prefer to feed on leaves over fruit, however defoliation can cause immature fruit, and sun burn. There are natural controls for the western grapeleaf skeletonizer, including parasitic wasps, and a host -specific granulosis virus. Management with chemical insecticides is also possible.

Cut worms

The most common cutworm species that attack grapes in North America are the spotted cutworm (*Amathes c-nigrum*), the variegated cutworm (*Peridroma saucia*), and the brassy cutworm (*Orhodes rufula*). Cutworms are inconspicuous Lepidopteran caterpillars which feed on new buds and young shoots. Injury to buds containing future canes and infloresence can cause reduction in yield. There are many natural enemies to cut worms, however there are few chemical pesticides available for control of cutworms.

Grape bud Beetle (*Glyptoscelis squamulata*)

Grape bud beetles feed on opening buds containing inflorescence and shoots, causing fruit loss and vine damage. Grape bud Beetle can be controlled by chemical insecticides.

Grape Leafhopper (*Erythroneura Elegantula*)
The Grape Leafhopper is a Homopteran insect that feeds on grapevine by sucking the sap from the leaves. Losses can occur from fruit spotting in table grapes, and from reduction in yield. Some cultivars can tolerate high populations of leafhopper and do not require treatment, however many vineyards require at least one pesticde aplication for the control of leafhopper each season.

Phylloxera (*Daktulosphaira vitifoliae*)
Phylloxera is a sap sucking insect similar to the aphid, and feeds on grape roots which causes stunted growth and eventually vine death. Grape Phylloxera are found throughout most all of California's grape-growing areas. The Phylloxera feeding sites impair absorption of nutrients, and water. Resistant rootstocks and environmental conditions are of great importance, for there is no well documented control of Phylloxera, particularly in heavy soils.

Nematodes
Nematodes are microscopic roundworms that feed on microorganisms or plant roots. Most vineyard soil contains nematodes feeding on grape roots, as well as other plants and organisms. Roots infected with nematodes are often unable to meet the plants demand for nutrients. Vines infected with plant parasitic nematodes display reduced vigor and yield, however vine death rarely occurs from nematode stress alone. Currently there is no solid method for controlling nematode populations other than the preplant fumigation with methyl bromide (which will soon be unavailable).

Our aim at Dry Creek Labs is to provide established cultivars which are genetically improved through the introduction of disease and/or pest resistance genes. This program involves the genetic transformation of clonal grapevine rootstock and scion varieties with insect resistance (Bt, GNA, Cowpea Trypsin Inhibitor) as well as fungal and bacterial resistance genes (lytic peptides). In this report we discuss the genetic transformation of three popular grapevine rootstocks with the GNA (Hilder. 1992) gene, using *Agrobacterium*-mediated transformation. The GNA gene has been reported to be effective in the control of Homopteran (sap-sucking) insects (patent application No. PCT/GB 92/01565) as well as nematodes (patent application 94 06371.6).

MATERIALS AND METHODS

Somatic embryos were induced from leaves of the grapevine cultivars Freedom, 101-14, and Teleki 5C (fig.1.). Proliferating embryos (fig.2.) were cocultivated with agrobacterium strain LBA4404 carrying the binary vector p1GNA2, which contains the ß-glucuronidase(GUS), GNA, and neomycin phosphotransferase II (NPTII) genes. Infected embryos were placed on selection media containing kanamycin for the selection of putative transgenic embryos, and Cefotaxium for the eradication of the *A. tumefaciens*. Kanamycin resistant embryos were allowed to proliferate on several rounds of selection, and germinate in the presence of kanamycin. Putative transformed grapevine clones were assayed for GUS activity with X-Gluc. Total cellular protein was extracted from the roots of non-transformed control plants, as well as transgenic clones, and relative concentrations of GNA protein were determined by Western blot analysis. DNA analysis were performed using southern blot hybridization. Clones were propagated for greenhouse studies and bioassays.

Fig. 1. Somatic embryogenesis from clonal 101-14 grapevine leaf explants

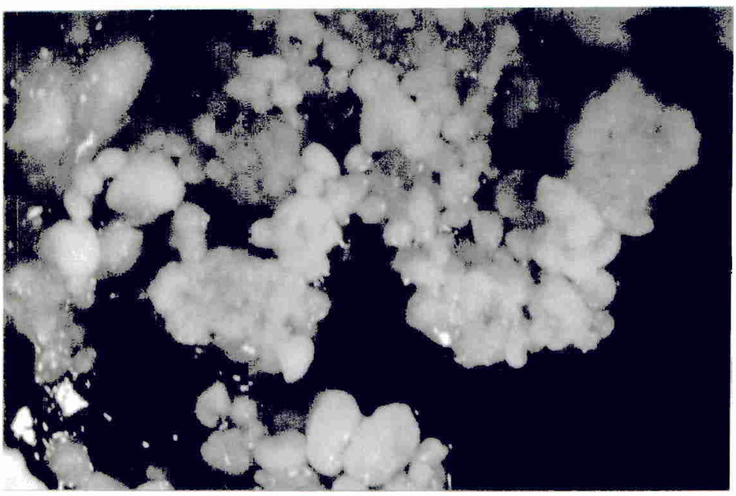

Fig.2. Proliferating clonal Freedom somatic embryos

RESULTS AND DISCUSSION

Over 100 individual transgenic clones were produced from a series of 12 experiments. The presence of the GUS enzyme in the putative clones was confirmed by incubation of embryos with X-Gluc (fig.3.). Western blot analysis for the GNA protein (fig.4) showed zero banding for GNA protein in control samples, whereas transgenic samples showed banding relative to the GNA protein standard depending on the clone. GNA protein concentrations were calculated between 0.16-0.40% of total cellular protein depending on the individual clone. Southern blot hybridization for the GNA gene confirmed the insertion

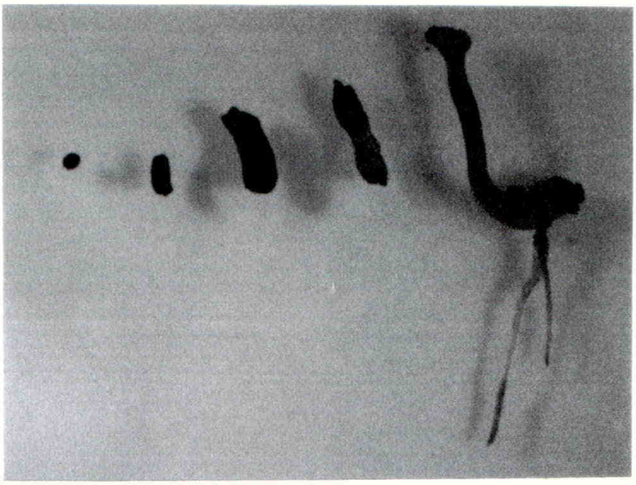

Fig. 3. Freedom embryos stained with X-Gluc

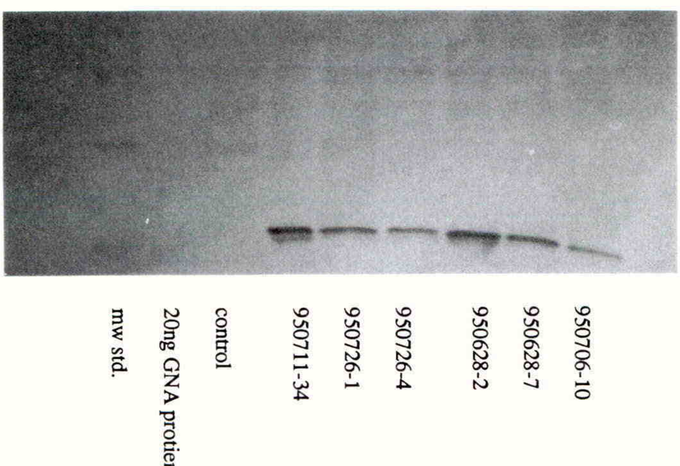

Fig. 4. Western blot of 50µg of protein extacted from roots of 6 transgenic clones and one non transformed control plant.

of the GNA gene into the grapevine plant geneome. Confirmed transgenic plants were micro-propagated in *vitro* and potted in soil for further studies (fig.5.and 6). We currently have over 50 clones containing the GNA gene involved in bioassays and greenhouse studies, and expect to obtain favorable data from these studies.

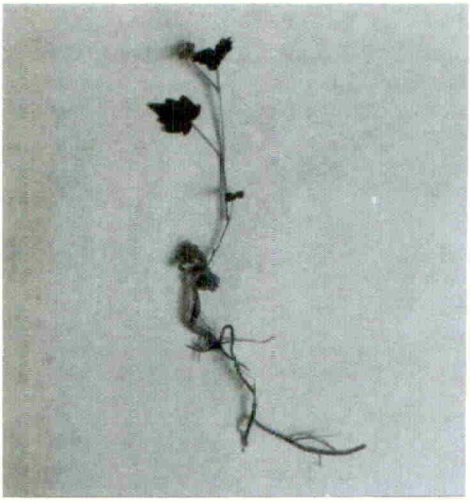

Fig. 5. Trangenic clone germinating from somatic embryo.

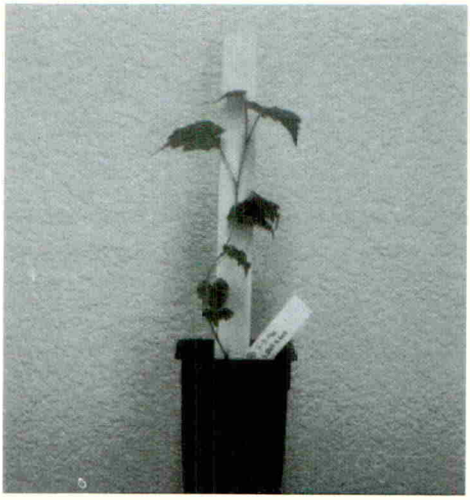

Fig. 6. Potted transgenic grapevine containing the GNA gene.

Recent advances have been made in the assembly of genetic constructs which contain the GNA translationally fused to ultra high expressing promoters such as the Ubi3(Garbarino 1994) and the Ubi7(Garbarino 92). We anticipate having data on grape clones containing such transgenes soon.

As viticulturists in North America face a diminishing list of approved chemicals for the management of grape pests and diseases, there is increasing concern about pest and disease management. Genetic engineering offers friendly solutions to many of these potential problems, while maintaining clonal cultivar identity.

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RESISTANCE OF *BOTRYTIS CINEREA* TO FUNGICIDES AND STRATEGIES FOR ITS CONTROL IN THE CHAMPAGNE VINEYARDS

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ABSTRACT

Strains of *Botrytis cinerea* resistant to dicarboximides, benzimidazoles and/or phenylcarbamates are commonly found in the Champagne vineyards. Limitations in the use of these fungicides combined with the application of other types of botryticides such as multi-site toxicants, anilinopyrimidines and phenylpyrroles are recognized as anti-resistance strategies.

INTRODUCTION

Grey mould caused by *B.cinerea* is one of the most damaging diseases of grapevine. Several prophylactic measures including limitation of nitrogen fertilizer, removal of leaves around bunches and correct control of grapevine moths, can reduce the impact of this fungal disease. However the main way to protect the grapevine against grey mould is the use of botryticides. Four preventive applications are traditionally recommended, at the end of flowering (stage A), at bunch closing (stage B), at veraison (stage C) and three weeks before harvest (stage D) ; this last treatment is rarely justified. Even if the registration procedures of a botryticide require these four treatments, it is not usually recommended because of resistance phenomena and/or risks of exceeding maximum residue level values. Facing these problems the vinegrowers have to manage the use of the various families of botryticides. In this paper we will describe the situation encountered in Champagne vineyards with multi-site fungicides (mainly thiram), antimicrotubules toxicants (mainly carbendazim and diethofencarb), dicarboximides (e.g. iprodione, procymidone, vinclozolin), phenylpyrroles (e.g. fludioxonil) and anilinopyrimidines (e.g. pyrimethanil).

MATERIALS AND METHODS

The effect of fungicides on the mycelial growth of *B.cinerea* was studied using the method of Leroux *et al.*, (1992). The nutrient medium contained : 10g glucose, 1.5g K_2HPO_4, 2g KH_2PO_4, 1g $(NH_4)\ 2SO_4$, 0.5g $MgSO_4.7H_2O$, 2g yeast extract and 12.5g agar for 1 litre ; yeast extract was not added in anilinopyrimidine tests (Leroux & Gredt, 1995). The inoculation of these media amended by various fungicide concentrations was realized with 5mm mycelial plugs and incubation took place at 18-20° C. The mycelial growth rates were evaluated by measuring the diameter of colonies during one week and the EC50 values were estimated (Leroux *et al.*, 1992).

Fungicide resistance in *B.cinerea* was usually monitored at grape harvest. Approximately 15-25 diseased berries from different bunches were collected at random throughout the tested vineyard and then dipped all together in sterile water ro rinse out the fungal conidia. The resulting spore suspension was adjusted to a concentration of 200.000 spores per ml and inoculated onto a glucose-agar medium (Leroux & Clerjeau, 1985) ; phosphate salts were added in pyrimethanil tests (Leroux & Gredt, 1995). The discriminatory concentrations of the various fungicides are mentioned in Table 2. After an incubation time of 18-24h at 18°C in the dark, spore germination and germ tube lenghts were evaluated under the microscope. From these observations the frequency of various phenotypes could be determined (Leroux & Clerjeau, 1985 ; Leroux & Gredt 1995).

Three trials were conducted in 1995 on cultivars Pinot Meunier or Pinot noir. Each experimental plot contained 240 to 423 vines and covered an area between 150 and 500 m2. Botryticides were applied at stages A, B or C and the volume of water was about 400 litres/ha. At the harvest the level of attack by *B.cinerea* was evaluated from 200 bunches.

RESULTS

Characteristics of the fungicide-resistant strains

The fungitoxicity (inhibition of hyphal growth) of benzimidazoles and phenylcarbamates is due to their binding to tubulin, the main protein of cellular microtubules. Two types of benzimidazoles-resistant strains are commonly found in Champagne vineyards. The Rb1 strains highly resistant to carbendazim and benomyl are susceptible to the phenylcarbamate diethofencarb, whereas the Rb2 ones which are moderately resistant towards benzimidazoles are totally insensitive to diethofencarb (Table 1). According to Yarden & Katan (1993) these benzimidazole-resistant strains differ from wild-type strains by single pair mutations of the gene encoding for beta-tubulin. In Rb1 alanine replaced glutamic acid (codon 198) whereas, in Rb2 tyrosine replaced phenylalanine (codon 200). These three phenotypes [wild-type, Rb1, Rb2] can be easily discriminated in field samples by using carbendazim alone or in mixture with diethofencarb (Table 2).

Table 1. Effects of fungicides on the mycelial growth of various strains of *Botrytis cinerea*

Fungicides	Mean EC50 values (mg/litre)					
	wild-type	resistant phenotypes [1]				
		Rb1	Rb2	Rd1	Rd2	Ra
benomyl	0.03	>50	5	-	-	-
carbendazim	0.02	>50	4	-	-	-
diethofencarb	>50	0.02	>50	-	-	-
iprodione	0.2	-	-	2.0	>50	-
procymidone	0.15	-	-	1.5	>50	-
vinclozolin	0.2	-	-	2.5	>50	-
fludioxonil	0.005	-	-	0.006	>5	-
cyprodinil	0.01	-	-	-	-	2
pyrimethanil	0.07	-	-	-	-	4

[1] All the resistant strains were from Champagne vineyards except Ra strains which came from Bordeaux region and Rd2 which were laboratory mutants

Strains of *B.cinerea* highly resistant to dicarboximides [Rd2] are easily induced in the laboratory but naturally remain exceptional in the nature (Gouot, 1988) ; such Rd2 strains in Champagne vineyards were never detected. The strains which provoke practical resistance towards dicarboximides are moderately resistant [Rd1] to iprodione, procymidone and vinclozolin (resistance levels about 10 ; Table 1). By using 10 mg/litre of procymidone or 5 mg/litre of vinclozolin it is easy to distinguish these Rd1 strains from the wild-type ones.

Higher concentrations of iprodione (30 to 40 mg/litre) allow to detect the possible Rd2 strains (Table 2 ; Leroux & Clerjeau, 1985).

Fludioxonil is a novel phenylpyrrole whose structure is related to that of fenpiclonil and of pyrrolnitrin, an antibiotic produced by several bacteria species. It was introduced in French vineyards in 1995, with interval harvest of 45 days. These phenylpyrroles, like dicarboximides, induce typical morphological alterations of germ tubes (i.e. swelling, branching, bursting) and they exhibit positive cross resistance in Rd2 srains (Faretra & Pollastro, 1993 ; Leroux *et al.*, 1992). However the dicarboximide-resistant strains of *B.cinerea* commonly found in vineyards [Rd1] remain sensitive to fludioxonil (Table 1). So, in practice, dicarboximides and phenylpyrroles must be considered as two distinct groups of botryticides. According to Faretra & Pollastro (1993), fungicide-resistance in Rd1 and Rd2 strains is determined by two alleles of the Daf 1 gene. These authors do not exclude the existence of other alleles of the Daf 1 gene or other genes, able to determine field-resistance to phenylpyrroles. To evaluate this risk, we monitored *B.cinerea* populations in the presence of 2.5 mg/litre of fenpiclonil or 1.0 mg/litre of fludioxonil (Table 2). The analyses conducted since 1993 did not reveal the existence of any phenylpyrrole-resistant strains.

Table 2. Fungicide concentrations used to characterize the various phenotypes of *B.cinerea* within spores populations

Fungicides	concentrations (mg/litre)	Responses of various phenotypes [1]					
		wild-type	Rb1	Rb2	Rd1	Rd2	Ra
carbendazim	1	-	+	+			
carbendazim	10	-	+	-			
carbendazim + diethofencarb	1+10	-	-	+			
procymidone	10	-			+	+	
vinclozolin	5	-			+	+	
iprodione	30-40	-			-	+	
fludioxonil	1	-			-	+	
pyrimethanil	1-3	-					+

[1] - : absence or presence of short germ-tubes ; + : presence of long germ-tubes

Pyrimethanil is a novel botryticide introduced in french vineyards in 1994 ; it belongs to anilinopyrimidines together with cyprodinil and mepanipyrim which are also registered in Switzerland since 1995. Their antifungal effect could result from a block of the excretion of hydrolytic enzymes (Milling &Richardson, 1995) or an inhibition of methionine biosynthesis (Leroux *et al.*, 1996). Strains of *B.cinerea* highly resistant to anilinopyrimidines have been detected recently in a location of the Bordeaux region (Table 1 ; Leroux & Gredt, 1995) and also in a long-term trial in Switzerland (Hilbert & Schuepp, 1996) ; in the last situation, practical resistance to anilinopyrimidines was observed. By using a single concentration of pyrimethanil between 1 and 3 mg/litre it is easy to detect Ra strains within *B.cinerea* populations (Table 2 ; Leroux & Gredt, 1996). Such a monitoring conducted in Champagne area since 1993 did not reveal the presence of any anilinopyrimidine-resistant strains.

Evolution of fungicide-resistance in Champagne

Every year, at vintage, a monitoring of fungicide-resistance is conducted in about 100 commercial Champagne vineyards. In the case of dicarboximides the generalization of the Rd1 strains in the early 1980s (due to their intensive use) led to their withdrawal in 1983. Subsequently, in the absence of dicarboximides sprays, the frequency of Rd1 strains decreased. This decline suggests that resistance to dixarboximides does not persist in the absence of selection pressure. Such a situation permitted their reintroduction in 1986 and resistance remained stable when only one spray was applied per season (1986 to 1989). A clear increase in the frequency of Rd1 strains was noted when many vinegrowers treated twice a year (1990 to 1992). As a result, the use of dicarboximides was discontinued for a second time in Champagne (Table 3 ; Leroux & Moncomble, 1993).

Table 3. Evolution of percentages of *B.cinerea* resistant strains in Champagne region between 1982 and 1995

Years	Dicarboximides		Antimicrotubules		
	Sprays [1]	% Rd1	Sprays [2]	% Rb1	% Rb2
1982	4	87	0	85	0
1983	0	72	0	82	0
1984	0	42	0	76	0
1985	0	22	0	94	0
1986	0.5	21	0	97	0
1987	0.8	30	0.8	97	0
1988	0.8	22	1.5	95	2
1989	1.1	30	1.1	77	21
1990	1.6	37	0.9	52	46
1991	1.7	48	0.9	38	62
1992	1.5	56	0.2	54	46
1993	0	34	0	70	29
1994	0.2	31	0.3	72	25
1995	0.4	28	0.4	69	29

[1] Average number of annual sprays with dicarboximides (alone or in mixture with thiram)

[2] Average number of annual sprays with the mixture carbendazim + diethofencarb

Due to the development of Rb1 strains, benzimidazoles were withdrawn in 1975. Subsequent monitoring indicated that frequencies of Rb1 strains remained high in spite of absence of benzimidazoles sprays for more than five years (Leroux & Clerjeau, 1985). This observation suggests that Rb1 strains do not exhibit reduced competitiveness in comparaison to wild-type strains. Benzimidazoles (in fact carbendazim) were reintroduced in 1987 as a mixture with diethofencarb. After only two seasons of use of the mixture carbendazim + diethofencarb, strains simultaneously resistant to both these fungicides [Rb2] were detected. In spite of the limitation to one spray per year, the frequency of Rb2 strains increased strongly in 1990 and 1991 and this led to the withdrawal of the mixture carbendazim + diethofencarb in 1993. The monotoring conducted in Champagne between 1992 and 1994, as well as long-term trials, indicated that resistance of type Rb2 decreased when the selection pression was released (Table 3 ; Leroux & Moncomble, 1993). Consequently, a discontinuous use of the mixture carbendazim + diethofencarb must be considered.

Chemical control of grey mould in practice

According to the evolution of fungicide-resistance and the introduction of new botryticides, the spray programmes against grey mould evolved continuously. In the last 10 years correct efficacies were achieved between 1987 and 1990 after the introduction of the mixture carbendazim + diethofencarb and more recently in 1995 with the use of fludioxonil and pyrimethanil (Table 4). From the trials conducted in 1995, it appeared that the tested programmes exhibited efficacy between 46 and 60%. Regarding the evolution of fungicide-resistance, it has been confirmed that the selection pressure exerted by one dicarboximide spray was lower than that of the mixture carbendazim + diethofencarb (Table 5).

Table 4. Evolution of the efficacy of the antibotrytis programmes in Champagne vineyards between 1986 and 1995

Years	Programmes (stages)			
	A	B	C	Efficacy [1]
1986	multi-site	multi-site	dicarboximides [2]	+
1987	multi-site	dicarboximides	carbe + dietho	++
1988	carbe + dietho	carbe + dietho	dicarboximides	++
1989	carbe + dietho	dicarboximides	dicarboximides	++
1990	carbe + dietho	dicarboximides	dicarboximides	++
1991	carbe + dietho	dicarboximides	dicarboximides	-
1992	multi-site	dicarboximides	dicarboximides	-
1993	-	-	-	(-)
1994	carbe + dietho carbe + dietho	multti-site pyrimethanil	pyrimethanil dicarboximides	+
1995	carbe + dietho fludioxonil	fludioxonil pyrimethanil	pyrimethanil dicarboximides	++

[1] Levels of efficacy ; - <25% control ; + 25-50% ; ++ >50%

[2] Dicarboximides are generally applied alone at stage C whereas they can be used in mixture with thiram at stage B

Table 5. Efficacy of various programmes against *B.cinerea* in three trials conducted in 1995

Programmes (stages) [1]			Efficacy	% resistance	
A	B	C	(% control)	Rd1	Rb2
fludioxonil	pyrimethanil	procymidone	53	33	8
carbendazim + diethofencarb	fludioxonil	procymidone	60	33	30
carbendazim + diethofencarb	pyrimethanil	procymidone	46	37	34
carbendazim + diethofencarb	fludioxonil	pyrimethanil	60	21	32
-	-	-	[24][2]	17	7

[1] The dosages were : fludioxonil 500 g/ha ; pyrimethanil 1000 g/ha ; procymidone 750 g/ha ; carbendazim + diethofencarb 500 + 500 g/ha

[2] Percentage of grey mould in the control plots at vintage

CONCLUSION

Today, the French vinegrowers may use five groups of botryticides (i.e. multi-site fungicides, antimicrotubules toxicants, dicarboximides and anilinopyrimidines). However, their utilization is restricted because of resistance phenomena, negative effects on fermentation (i.e. multi-site, fungicides) or the presence of residues. None of them can be recommended alone throughout the season (as was the case in the past with benzimidazoles or dicarboximides). The present strategy in Champagne area consists in treating vineyards at stages A, B or C with three different botryticides. The recommendation is to use fludioxonil, pyrimethanil and to complete with either a dicarboximide or the mixture carbendazim + diethofencarb. (these antimicrotubule fungicides should not be applied every year on the same plot). The alternation of the various groups of botryticides with a maximum of one spray per year for each family has been adopted in all French vineyards. The development of mixtures of products with different biochemical modes of action (e.g. fludioxonil + cyprodinil) could be another approach towards resistance managment against grey mould (Hilber & Schüepp, 1996).

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EFFICACY OF STROBILURINE DERIVATIVES AGAINST GRAPE POWDERY MILDEW IN NORTHERN ITALY

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ABSTRACT

The results obtained in four experimental trials carried out in Northern Italy against grape powdery mildew (*Uncinula necator*) are reported. The two strobilurine derivatives, kresoxym-methyl and azoxystrobin, performed well when applied as preventive treatments, respectively at 10 and 25 g a.i./100 litres. They significantly reduced grape powdery mildew, generally performing better than standard EBI fungicides. Their activity was strongly reduced when their application was delayed after development of symptoms. The strategies of their application are briefly outlined.

INTRODUCTION

Powdery mildew, caused by *Uncinula necator*, can result in very serious damage and crop losses on grapevine in Italy. Its attacks have been particularly severe during the past years in Northern Italy. Ergosterol biosynthesis inhibitor fungicides (EBIs) continue to be the most widely applied chemicals, together with sulphur, which still represents a large part of the grape powdery mildew market in Italy. EBIs still perform relatively well, despite the development of resistance to some of them, a phenomenon observed also in Italy since 1988 (Garibaldi *et al.*, 1990). During the last few years, two new fungicides, derived from the fungal secondary metabolite strobilurine, have been developed (Ammermann *et al.*, 1992; Godwin *et al.*, 1992). Both azoxystrobin and kresoxym-methyl show very good potential for being applied against grape powdery mildew as well as against other diseases (i.e. downy mildew).

The results obtained in experimental trials carried out in Northern Italy in 1994 and 1995, with the aim of evaluating the efficacy of the two strobilurine derivatives compared to that of commonly used EBIs and sulphur, are here reported.

MATERIALS AND METHODS

Four experimental trials have been carried out in 1994 and 1995 in Northern Italy, in vineyards located in Piedmont, Liguria and Emilia-Romagna, generally characterized by the presence of severe attacks of powdery mildew, as described under table 1, on three different cultivars (Moscato, Pigato and Sangiovese) with the aim of evaluating the effectiveness of strobilurine derivative fungicides against *U. necator*. The fungicides listed under table 2 were

applied, by means of a knapsack sprayer, at the dosages reported under tables 3-6. Treatments started at the very first appearance of symptoms or at a later stage, in trials 1 and 2, when curative activity of azoxystrobin was evaluated.
Treatments were arranged using a randomized block design, with the number of replicates reported under table 1. At regular intervals, the percentages of infected bunches and berries were evaluated. Data were statistically analyzed by applying variance analysis and Duncan's Multiple Range test.

RESULTS

Disease incidence was quite high in 1994 at both sites, and less severe in 1995. In trial 1 (table 3), all tested fungicides significantly reduced powdery mildew incidence. The best efficacy was shown by kresoxym-methyl when applied at 10 g/100 l, in 8 sprays, in the presence of a very high disease pressure. It controlled powdery mildew almost completely. Also triadimenol+sulphur performed well, when applied in 7 sprays. A statistically similar level of protection was shown by azoxystrobin, when applied in 8 sprays at 25 g/100 l. The same fungicide showed a significantly lower level of disease control when applied 8 times at a lower rate (18.75 g/100 l) and even less when applied 9 times at 12.5 g/100 l. Azoxystrobin, however, controlled powdery mildew very poorly when the first spray was applied at a later stage, after the initial development of symptoms. This was due to the fast spread of the epidemic under conditions very favourable to pathogen development (table 3).
In trial 2 (table 4), again in the presence of a high disease pressure, the best results were obtained with an early start of sprays with sulphur, applied alone in 7 sprays or applied in the two earlier sprays, followed by hexaconazole (4 sprays). Both azoxystrobin (at 18.75 or 25 g/100 l) and kresoxim-methyl (at 10 g/100 l), applied at the appearance of the very first symptoms (end of flowering) performed well and similarly when applied in four treatments. Azoxystrobin was also effective at 12.5 g/100 l, when applied in 5 sprays. Slightly lower, but statistically not different, was the disease control provided by hexaconazole alone, applied in 4 sprays. Disease control was higher when hexaconazole treatments were preceded by sprays of sulphur.
Although in the presence of a much lower disease pressure, a similar trend was observed at both sites in 1995. In 1995 all treatments started at the very beginning of symptoms development and all the tested fungicides performed well (tables 5 and 6).

In conclusion, strobilurine derivatives performed well and, in some cases (i.e. trial 2), significantly better than the standard EBI fungicide, when applied early as a preventative strategy. The results obtained at Albenga in 1994 suggest a better activity of kresoxym-methyl than azoxystrobin. Such results, however, will need to be confirmed under similar disease pressure.
Also, the results obtained indicate that both strobilurine derivatives should be applied as preventative sprays or immediately after the very first symptoms develop. A delayed application of the first treatment, particularly in the presence of a very severe disease epidemic, as that observed at Albenga in 1994, led to very unsatisfactory results. It must be stressed that the latter approach was used only for experimental purposes. The results reported here confirm those previously obtained by applying EBIs fungicides after the development of first symptoms (Brunelli *et al.*, 1992; Monchiero *et al.*, 1992) and suggest once more that such a strategy of disease control is not applicable in areas where severe powdery mildew epidemics occur commonly. Actually, in the 1994 trials, early application,

Table 1. Layout of the four experimental trials

	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4
Site	Albenga (Savona)	Borghi (Forli')	Borghi (Forli')	Cessole (Asti)
Variety	Pigato	Sangiovese	Sangiovese	Moscato
Year	1994	1994	1995	1995
Age (years)	20	20	20	15
Growing system	Guyot	arched cane	arched cane	Guyot
Volume/ha (l)	800	1,000-1,800	1,000-1,800	500-700
Vines/plot	20	6	6	10
N. replicates	3	4	4	4
Flowering: start	27/5	23/5	1/6	30/5
end	6/6	2/6	15/6	10/6
First symptoms	27/5	30/5	28/6	1/7

Table 2. List of fungicides used in the different trials

FORMULATE	% a.i.	ACTIVE INGREDIENT	COMPANY	YEAR OF APPLICATION
ANVIL SC	22.9	Hexaconazole	Solplant	1994-1995
BAYFIDAN COMBI WP	2.5+50	Triadimenol+sulphur	Bayer	1994
BAS 490 F EC	25	Kresoxym-methyl	BASF	1994 - 1995
ICI 5504 9246 SC	25	Azoxystrobin	Solplant	1994 - 1995
MICROTHIOL WG	80	Sulphur	ELF Atochem	1994
SISTHANE E	13.4	Myclobutanil	Rhone-Poulenc	1995
TIOVIT WG	80	Sulphur	Sandoz	1995

Table 3. Efficacy of different fungicides against grape powdery mildew on the cv Pigato (trial 1, Albenga, 1994)

TREATMENT	g a.i./100l	N. SPRAYS	% INFECTED BERRIES JUNE 17	JUNE 30	JULY 27	AUGUST 9
--	--	--	33.9 e ^	78.2 d	86.2 e	87.5 e
Azoxystrobin	25.00	8*	5.4 bc	4.0 a	19.3 bc	19.2 a
Azoxystrobin	25.00	6**	28.7 d	50.6 c	70.5 e	61.5 d
Azoxystrobin	18.75	8*	7.2 c	15.6 ab	35.0 cd	37.3 b
Azoxystrobin	12.50	9***	7.2 c	29.2 bc	47.6 d	48.0 c
Azoxystrobin	12.50	8*	8.7 c	38.0 bc	50.9 d	57.3 c
Kresoxym-methyl	10.00	8*	2.8 ab	0.21 a	0.6 a	0.1 a
Kresoxym-methyl	10.00	6**	25.9 d	44.8 c	53.5 d	60.8 d
Triadimenol+sulphur	5.0+10.0	7°	1.3 a	2.9 a	10.3 ab	11.6 a

^ The values of the same column, followed by the same letter, do not significantly differ, following Duncan's Multiple Range Test (P = 0.05)

Dates of spray:

* 6/5; 19/5; 31/5; 9/6; 20/6; 1/7; 11/7; 21/7 (first spray: before flowering)

** 27/5; 6/6; 15/6; 23/6; 5/7; 15/7 (first spray: beginning of flowering)

*** 6/6; 19/5; 27/5; 6/6; 15/6; 23/6; 1/7; 11/7; 21/7 (first spray: end of flowering)

° 6/5; 19/5; 31/5; 9/6; 20/6; 5/7; 15/7 (first spray: before flowering)

Treatments against downy mildew: Ridomil R (metalaxyl + copper oxychloride 4+40%) on 18/4; 21/5

Table 4. Efficacy of different fungicides against grape powdery mildew on the cv Sangiovese (trial 2, Borghi, 1994)

TREATMENT*	g a.i./100 l	SPRAYS CARRIED OUT AT	% INFECTED BERRIES JULY 4	AUGUST 9
--	--	--	80.5 c ^	81.6 c
Azoxystrobin	25.00	7/6; 20/6; 12/7; 25/7	3.1 ab	7.4 ab
Azoxystrobin	18.75	7/6; 20/6; 12/7; 25/7	4.9 ab	6.7 ab
Azoxystrobin	12.50	7/6; 20/6; 12/7; 25/7	5.6 ab	9.0 ab
Azoxystrobin	12.50	7/6; 16/6; 24/6; 12/7; 25/7	2.7 ab	3.4 ab
Kresoxym-methyl	10.00	7/6; 20/6; 12/7; 25/7	3.6 ab	3.1 ab
Hexaconazole	2.10	7/6; 20/6; 12/7; 25/7	9.0 b	9.8 b
Sulphur	400.00	23/5; 2/6	1.8 a	3.9 ab
Hexaconazole	2.10	7/6; 20/6; 12/7; 25/7		
Sulphur	400.00	23/5; 2/6; 7/6; 16/6; 24/6; 12/7; 25/7	1.1 a	1.9 a

* Treatments against powdery mildew from end of flowering. Two sprays before flowering with sulphur.

Other treatments: 5/7: sulphur dust, 30 kg/ha on all plots

Treatments against downy mildew: Ridomil MZ (metalaxyl+mancozeb 8+64%): 7/6; 20/6

^ see table 1

Table 5. Efficacy of different fungicides against grape powdery mildew on the cv Sangiovese (trial 3, Borghi, 1995).

TREATMENT*	g a.i./100 l	SPRAYS CARRIED OUT AT	% INFECTED BUNCHES (AUGUST 8)	% INFECTED BERRIES (AUGUST 8)
--	--	--	35.1 b ^	3.8 b
Azoxystrobin	25.00	1/6; 15/6; 29/6; 13/7; 27/7	0.0 a	0.0 a
Azoxystrobin	18.75	1/6; 15/6; 29/6; 13/7; 27/7	0.2 a	0.0 a
Azoxystrobin	12.50	1/6; 15/6; 29/6; 13/7; 27/7	0.0 a	0.0 a
Kresoxym-methyl	15.00	1/6; 15/6; 29/6; 13/7; 27/7	0.0 a	0.0 a
Kresoxym-methyl	10.00	1/6; 15/6; 29/6; 13/7; 27/7	0.0 a	0.0 a
Hexaconazole	2.10	1/6; 15/6; 29/6; 13/7; 27/7	0.0 a	0.0 a
Sulphur	400.00	8/5; 22/5; 1/6; 9/6; 15/6; 26/6; 5/7; 13/7; 22/7	1.7 a	0.0 a

* Treatments against powdery miildew carried out starting at the end of flowering. Two sprays (with sulphur) before flowering.
Treatments against downy mildew: Ridomil MZ: 29/5; 12/6
^ see table 1

Table 6. Efficacy of different fungicides against grape powdery mildew on the cv Moscato (trial 4, Cessole, 1995).

TREATMENT*	g a.i./100 l	SPRAYS CARRIED OUT AT	% INFECTED BUNCHES (JULY 7)	% INFECTED BERRIES (JULY 30)
--	--	--	38.0 b ^	5.9 b
Azoxystrobin	25.00	29/5;12/6;24/6;7/7; 21/7	1.0 a	0.0 a
Azoxystrobin	18.75	29/5;9/6;19/6;28/6;7/7; 16/7; 26/7	2.0 a	0.0 a
Azoxystrobin	18.75	29/5;12/6;24/6;7/7; 21/7	6.3 a	0.1 a
Azoxystrobin	12.50	29/5;9/6;19/6;28/6;7/7; 16/7; 26/7	9.0 a	0.7 a
Kresoxym-methyl	15.00	29/5;12/6;24/6;7/7; 21/7	0.5 a	0.0 a
Kresoxim-methyl	10.00	29/5;9/6;19/6;28/6;7/7; 16/7; 26/7	1.8 a	0.0 a
Kresoxym-methyl	10.00	29/5;12/6;24/6;7/7; 21/7	0.0 a	0.0 a
Myclobutanil	6.70	29/5;12/6;24/6;7/7; 21/7	0.0 a	0.0 a

* Treatments against powdery mildew start of flowering
Treatments against downy mildew: Ridomil MZ: 29/5; 12/6
Curzate M (cymoxanil + mancozeb 4+40):15/5;19/6;28/6
Curzate R (cymoxanil +Cu oxuychloride 4.2+39.75):7/7; 16/7; 26/7

^ see table 1

before flowering, achieved a very good result, even with sulphur alone. Moreover, a control strategy based on a delayed application of the first treatment is risky, since it could easily favour the selection of resistant strains in the population of the pathogen.

Having established the good performance of the two strobilurine derivatives against grape powdery mildew under Italian environmental conditions, further experimental trials are needed in order to evaluate how to utilise a limited number of sprays per season (3-4) of the new chemicals within an effective and practical disease control strategy.

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