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Effects of vapours of chlorpropham and ethofumesate on wild plant species

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"Capsule": *Non-target species responded to low concentrations of herbicide vapours in a fumigation study with foliar injury being the most responsive parameter.*

Abstract

Effects of vapours of two herbicides on plantlets of fourteen wild higher plant species and two bryophytes were screened in fumigation experiments using foliar injury, chlorophyll fluorescence and growth as response parameters. After vaporisation of the herbicides for 48 h, concentrations in the chambers reached 77 μg m^{-3} in the chlorpropham treatments and 184 ng m^{-3} in the ethofumesate treatments. Despite the higher concentrations of the volatile chlorpropham (vapour pressure, VP: 1.3 mP), plants showed no foliar injury, but vapours of this herbicide caused leaf crinkling in the agriophyte *Agrostemma githago*. The less volatile ethofumesate (VP: 0.56 mP) caused foliar injury in all higher species, with lowest no observed effect concentrations (NOECs) of 75 ng m^{-3}. Chlorpropham affected growth only in *Agrostemma*, while ethofumesate reduced growth in one third of the higher plant species. Chlorophyll fluorescence proved to be a less suitable response parameter compared to foliar injury and growth. No adverse effects were observed in mosses, probably due to the slow growth and hence small doses of herbicides taken up. The extent of foliar injury due to ethofumesate showed a weak positive relationship to relative growth rates and specific leaf area in the tested higher plant species.

Keywords: Natural vegetation; NOEC; Non-target plants; Phytotoxicity; Risk assessment

1. Introduction

Environmental concern has recently arisen on potential impacts of air-transported agrochemicals and their impact on non-target organisms (Forster et al., 1995; Klöppel and Kördel, 1997; Guicheret et al., 1999; van Dijk et al., 1999). Adverse ecological effects are not restricted to the bordering habitats (field strips, hedgerows and ditches) alone. The remoter semi-natural ecosystems may also be affected by pesticide vapours and pesticide spray drift. Estimations and model studies on losses of pesticides to the atmosphere have shown that more than half of the applied compounds may be volatilised after field sprayings in the Netherlands (de Jong et al., 1994; Jansma and Linders, 1995; Polder et al., 1997; Smit et al., 1997). As a consequence of the specific Dutch agricultural acreage situation, the non-target natural vegetation in this country is exposed to an average of 0.02 equivalents of the recommended herbicide dose (Klepper et al., 1998). While economic losses due to vaporisation of herbicides are believed to be only minor, the adverse environmental effects of unwanted releases may not be acceptable.

Apart from spraying technology, pesticide fomulation and unfavourable climate conditions, the volatilisation of pesticides and the air transport of vapours depends on physiochemical characteristics of the compounds, including photostability, Henry's law constants and vapour pressures. Volatile substances may easily evaporate from spray solution, plant and soil surfaces, but their higher air concentrations do not necessarily create larger environmental risks than the usually lower air concentrations of less volatile compounds.

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In the future, the placing of new agrochemicals on the European market will have to be preceded by the evaluation of adverse effects on non-target organisms (EU Directive 91/414/EEC, November 1997), including animals, non-target crops and wild plant species. In order to assess the risk of pesticides to non-targets plants, tiered approaches have been presented by Aldrige et al. (1993), Füll et al. (1999) and Kördel et al. (1999) to be included in regulation procedures. All of these refer to higher plant species and neglect potential risks to lower plants like bryophytes and lichens, which also play a significant role in global biodiversity. The negative impacts of pesticides on non-target plant taxa, plant functional groups and ecosystem types have not yet been evaluated. Attempts to assess deposition of pesticides via the air, taking into account the build-up of higher concentrations of stable compounds in soils and water, must fail due to the scarce data on environmental concentrations.

A clear-cut differentiation between ranges of action of pesticide spray drift and pesticide vapours requires the costly monitoring of pesticides in the air and the development of methods to assess the impact of herbicides on non-target plants in the field. Using bioindicator plants, Marrs et al. (1993) and Davis et al. (1994) were the first to study the action radius of herbicides blown from a land parcel and the suitability of hedgerows to reduce blow-off. A multi-year floristic field study in a heavily used agricultural area in The Netherlands confirmed minor effects of herbicide use on the species composition of field-border habitats (de Snoo and van der Poll, 1999).

While pesticide impacts on non-target species are generally difficult to demonstrate and evaluate in the field, controlled experiments can be performed under laboratory conditions to derive effective concentrations (EC) of phytotoxic herbicide vapours in the air. These approaches make use of the airflow method, which has been introduced by Breeze et al. (1992) and Breeze (1993) in a study of the effects of agrochemicals on non-target species. Since then, more fumigation experiments have been performed to determine volatilisation rates of herbicides and the no observed effect concentrations (NOECs) of herbicide vapours on a variety of plant species, including plants from the native European flora (Walter et al., 1996; Walter et al., 1997; Frost et al., 1997; Schweizer and Hurle, 1999; Schweizer et al., 2000).

In this paper, we present results of a fumigation experiment in which a number of higher and lower plant species were exposed to vapours of the herbicides ethofumesate and chlorpropham. The aim of the study was to derive effective vapour concentrations of the two herbicides, to screen a wide range of wild plant species from different functional groups and to identify suitable response parameters for the assessment of adverse effects.

2. Material and methods

2.1. Choice of plant species and cultivation

The names and biological characteristics of plant species used in the experiments are given in Table 1; this table also summarises information on taxonomy, habitat preference and ecology of the species. Data on relative growth rate (RGR) and specific leaf area (SLA)

Table 1
Taxonomy, habitat, ecology, relative growth rates (RGR) and specific leaf area (SLA) of plant species used in the fumigation experiments

Plant species	Plant family	Natural habitat	Ecological strategy[a]	RGR[b] (mg g^{-1} d^{-1})	SLA[b] (m^2 kg^{-1})
Achillea millefolium	Asteraceae	Field boundary	C	241	26
Achillea ptarmica	Asteraceae	Wetlands	CS	238	37
Agrostemma githago	Caryophyllaceae	Ruderal sites	CR	153	n.i.[c]
Cirsium dissectum	Asteraceae	Wetlands	CSR	154	20
Eupatorium cannabinum	Asteraceae	Hedges, ditches	C	176	36
Molinia caerulea	Poaceae	Wetlands	CS	87	20
Hypericum perforatum	Guttiferae	Field boundary	C	205	50
Lychnis flos-cuculi	Caryophyllaceae	Ditches	CSR	204	35
Trifolium repens	Fabaceae	Grasslands	CSR	206	40
Species only *screened for foliar injury*					
Calendula arvensis	Asteraceae	Ruderal sites	R	n.i.	n.i.
Carex nigra	Cyperaceae	Ditches, wetlands	CS	147	33
Hyoscyamus niger	Solanaceae	Ruderal sites	CR	n.i.	n.i.
Lysimachia vulgaris	Primulaceae	Ditches	CSR	223	42
Lythrum salicaria	Lythraceae	Ditches	CS	197	44
Polytrichum formosum	Bryophytae	Forests	n.i.	n.i.	n.i.
Rhytidiadelphus squarrosus	Bryophytae	Ditches, forests	n.i.	n.i.	n.i.

[a] Refers to Frank and Klotz (1990); C, competitor; S, stress tolerator; and R, ruderal.
[b] After Hunt and Cornelissen (1997) and van der Werf et al. (1998).
[c] No information available.

were extracted from Hunt and Cornelissen (1997) and van der Werf et al. (1998). Information on ecological strategies of the species came from Frank and Klotz (1990). Their compilation is based on the CSR classification of Grime et al. (1988) differentiating between competitive species (C); slow-growing, long-lived stress tolerators (S) and fast-growing, short-lived ruderals (R).

Seeds of the higher plant species originated from field collections performed in the eastern part of The Netherlands and southwestern Germany. Soon after germination, young seedlings were transplanted into black plastic pots (340 ml) which were filled with a peat:sand mixture (2:1, v:v). Only *Carex nigra*, *Molinia caerulea* and *Lysimachia vulgaris* were propagated from mother plants originating from wetlands near Wageningen. Blankets of bryophytes and the underlying humus material were collected in a mature forest near Wageningen. The blankets were cut into equal pieces and material was filled into black plastic pots. Mosses were allowed to acclimatise to the greenhouse for several weeks before being used in the experiments. They were kept in a shaded area and sprayed with water every day. Plant cultivation and fumigation experiments were performed in a climate-controlled greenhouse. Night temperatures in this greenhouse were kept constant at 18°C, while day temperatures (7–21 h, Central European Time) were kept below 25°C. Relative humidity within the fumigation chambers was high (>80%) and photosynthetic active radiation (PAR) relatively low (<20 molE m^{-1} d^{-1}).

2.2. Choice of herbicides

Both pre-emergent herbicides were purchased from Luxan B.V. (Elst, The Netherlands). Ethofumesate was chosen because it is the most volatile herbicide of the ten herbicides with the highest sales volume in The Netherlands (CBS, 1995). The systemic herbicide is used in beet and inhibits the synthesis of fatty acids, the development of cuticles and the growth of meristems in a wide range of weeds, except of camomile. In a screening experiment of vapours of common herbicides, it had previously been reported to have high phytotoxicity in crop plants (Kempenaar et al., 1999). Chlorpropham was chosen as a second herbicide because vapours of this herbicide have been reported to cause damage to flowering crop plants in the Netherlands (Naber, 1989). The systemic herbicide is primarily used in onion cultures and inhibits root and epicotyl growth and the cell division of grasses.

2.3. Fumigation system and exposure of plants

An air flow-system was used to expose plant species to herbicide vapours under controlled conditions. The system consisted of a mass flow controller fixed to a tube through which compressed air was supplied. From the outlet of the mass-flow controller the adjusted air stream was blown into Erlenmeyer-flasks filled with aqueous herbicide solutions. Air turbulence in the flask caused gradual vaporisation of herbicides and vapours moved from the lower outlet of the flask via Teflon tubes into the closed exposure chambers (46×31×15 cm) where plants were kept. Contaminated air passed from the outlet of the chambers and left the greenhouse via tubes. The airflow in the system was adjusted to 6.4 l min^{-1}. Fig. 1 shows the layout of the fumigation system.

Five fumigation chambers were used in the experiments. Herbicide fumigation was conducted in replicate with 1:10 and 1:100 dilutions. The latter dilution is comparable to herbicide concentrations in field sprayings. According to the producers, field application rate for ethofumesate is 4 l ha^{-1} in 500 l water and for chlorpropham it is 2 l ha^{-1} in 300 l water. In order to simulate post application volatilisation, 50 ml of the diluted herbicide was filled into Erlenmeyer flasks, from which herbicides were allowed to evaporate for 48 h. One chamber was used as a control, i.e. the Erlenmeyer flask was filled with 50 ml water. The treatment position was at random.

2.4. Monitoring of herbicide air concentrations

Analyses were performed by TNO-MEP (Apeldoorn, The Netherlands, certification ISO9001). Air from the outlet of the fumigation chambers was passed through XAD-filled cartridges at a sampling rate of 120 l h^{-1}. Samples were taken at three different time intervals: 4–5, 24–25 and 48–49 h after the onset of fumigation. This

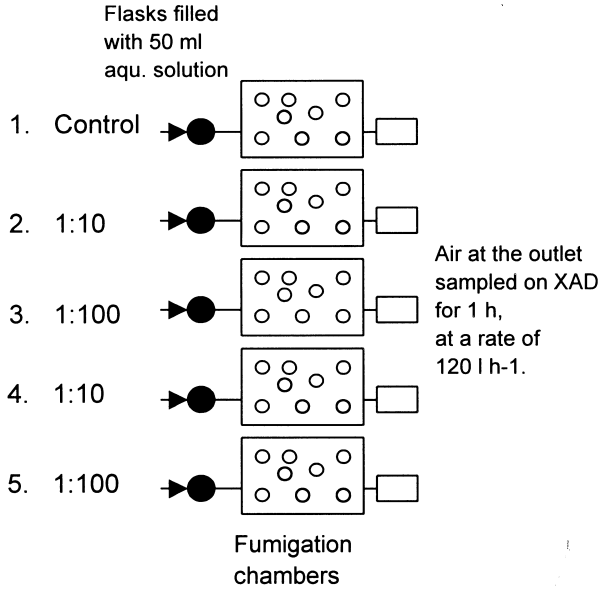

Fig. 1. Schematic representation of the "air flow system" used in the experiment. Five fumigation chambers were operated simultaneously; in each chamber eight plants were exposed to herbicide vapours. Flasks were filled with herbicide solutions at two dilutions and a stream of pressurised air was led through the system at a rate of 6.4 l min^{-1}.

was done for both herbicides and both treatments, i.e. dilutions of either 1:10 or 1:100. Cartridges were extracted with ethylacetate and samples were reduced nearly to dryness prior to analysis. Limits of detection were 0.05 μg per cartridge for chlorpropham and 0.005 μg per cartridge for ethofumesate. An external standard was used for quantification. Analyses were conducted by means of an HP 6890 gas chromatograph coupled to an HP 5793 mass spectrometer. Compounds were identified using their specific retention times and mass spectra.

2.5. Plant response parameters

Eight plants were placed in each of the five fumigation chambers and two plants at a time were taken out of the chambers after 4, 8, 24 and 48 h, respectively. After exposure, plants were randomly placed on a tray (100×100 cm) in a greenhouse and in the following 2 weeks plant development was closely monitored. At day 2 after exposure, chlorophyll fluorescence was determined according to Ketel and Lotz (1997), for which a portable plant photosynthesis meter (model PPM; EARS, 1998) was used; fluorescence at ambient light (F) and at light saturation (F_m) were measured. Light use efficiency (LUE) may be derived from these parameters after $\phi_P = (1-F/F_m)$.

At day 7 after termination of the exposure, foliar injury of plants was assessed, recording the total percentage of damaged leaf area. At day 14, plant height was measured and the aboveground biomass of the plants was harvested. Plant material was dried at 80°C for 48 h and dry weight was determined.

2.6. Statistical procedures

Descriptive and multivariate methods were used to address different sensitivities of the plant species. Analyses of variance were calculated to identify significant treatment effects on the response parameters visible injury, chlorophyll fluorescence, height and shoot dry weight. Experiments had a randomised block design, with concentration of the herbicide on the main plot and duration of the exposure on the sub-plot. Dose–response relationships were derived for shoot biomass of nine test species, while information on visible injury was available for 16 species.

3. Results

3.1. Concentrations of herbicides

Along with the evaporation of the aqueous solutions over time, air concentrations of herbicides gradually increased from low values after 4 h to about three times higher values after 48 h (Table 2). Two-day fumigation with 1:10 dilutions of herbicides produced maximum air concentrations of 184 ng m^{-3} for ethofumesate and high values of 77 μg m^{-3} for chlorpropham. The high air concentrations of chlorpropham are due to the high vapour pressure (1.3 mPa) of this compound, whereas ethofumesate (VP = 0.65 mPa) is less volatile. Maximum air concentrations were significantly lower in the treatments with a 1:100 dilution (Table 2).

3.2. Chlorophyll fluorescence

Effects of herbicide treatment on chlorophyll fluorescence were assessed in nine species. Untreated plants showed readings of about 70, which is common for unstressed plants, while readings below 15 normally indicate high mortality within a few days. A significant reduction of chlorophyll fluorescence due to ethofumesate was observed in the genera *Achillea* ($P < 0.05$), *Eupatorium cannabinum* ($P < 0.001$) and *Agrostemma githago* ($P = 0.031$) after exposure to 1:10 diluted herbicide vapours for 48 h. In the *Achilleas*, readings went down from 70 to 30, while in the other species, values were only slightly reduced from 70 to 60. In *Agrostemma* chlorophyll fluorescence was also significantly reduced by chlorpropham ($P < 0.001$): values dropped from 80 in plants from the control to 70 in plants exposed to vapours with the highest herbicide concentrations, i.e. at a concentration of 77 μg m^{-3}. The NOEC for a reduction in chlorophyll fluorescence is about 150 ng m^{-3} for ethofumesate vapours.

3.3. Plant growth

Effects of herbicide vapours on dry weights and heights were determined in nine species. Dry weights were significantly reduced by ethofumesate vapours in five species: *Achillea ptarmica* ($P = 0.012$), *Achillea millefolium* ($P = 0.003$), *E. cannabinum* ($P = 0.05$), *Lychnis*

Table 2
Herbicide concentrations determined in the air at different intervals of the fumigation experiment

Air sampling at hours	Chlorpropham [μg m^{-3}]		Ethofumesate [ng m^{-3}]	
	1:100 dilution	1:10 dilution	1:100 dilution	1:10 dilution
04–05	8.2	24.6	41.7	74.8
24–25	14.3	14.0	92.9	129.9
48–49	41.7	77.4	143.5	184.0

flos-cuculi ($P = 0.008$) and *Hypericum perforatum* ($P = 0.006$) after exposure to vapours of the 1:10 treatments for 48 h. The NOEC for a reduction in shoot biomass is thus about 150 ng m^{-3} for ethofumesate vapours. Chlorpropham vapours decreased shoot weight only in *Agrostemma githago* ($P < 0.001$). Growth reductions after longer exposure are due to the gradually increasing herbicide concentrations in the vapours. Ethofumesate vapours significantly decreased plant height only in *Achillea ptarmica* ($P = 0.001$) which was clearly related to leaf injury (loss of photosynthetically active leaf area) and hence, the reduction of growth caused by that herbicide. Highly significant reductions of plant height due to vapours of chlorpropham were observed only in *Agrostemma githago* ($P < 0.001$).

3.4. Foliar injury

Foliar injury at day 7 after the exposure was a much more sensitive response parameter than growth response or reduction of chlorophyll fluorescence at day 2. Sixteen species were screened for visible effects of herbicide vapours. Ethofumesate vapours from the 1:10 dilutions caused significant foliar injury in all higher plant species (Fig. 2), while visible injury was absent in plants treated with vapours of chlorpropham. The first injuries occurred at day 2 after the termination of the ethofumesate fumigation with strongest effects in *Hypericum perforatum*, the *Achilleas* and the agriophytes *Calendula* and *Hyoscyamus*. Characteristic bleaching symptoms (white flecks) due to ethofumesate appeared on the leaf tips first, and soon expanded into the centre of the leaves. A NOEC for foliar injury due to ethofumesate vapours of 75 ng m^{-3} can be derived from the dose-response relationship in Fig. 3.

The extent of foliar injury due to ethofumesate in the different species was related to the plant traits RGR and SLA (data in Table 1). While fast-growing species tend to show more foliar injury, leaf thickness (SLA) seems to play a minor role in the development of acute symptoms (Fig. 4). In contrast to ethofumesate, chlorpropham did not cause foliar injury in any of the tested species, but vapours of this herbicide affected leaf anatomy in *Agrostemma githago*. Plants exposed to high concentrations of chlorpropham for 48 h showed "leaf crinkling".

4. Discussion

Comparing the herbicide air concentrations in these experiments with concentrations of the herbicides in ambient air was not possible because both compounds, ethofumesate and chlorpropham, are not routinely monitored in the field and information on air concentrations of these herbicides was not available from the literature.

The development of foliar symptoms due to herbicide vapours proved to be a very reliable measure of a wild plant's sensitivity to herbicide vapours in our study. Although all higher species showed distinct foliar symptoms due to vapours of ethofumesate, reduction of growth and chlorophyll fluorescence was observed only in some taxa. While 48-h exposure to vapours of an undiluted ethofumesate solution in the study of

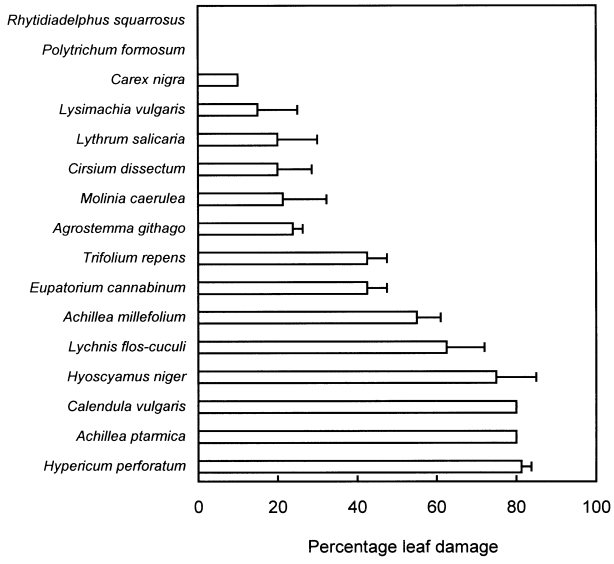

Fig. 2. Foliar injury (% leaf area per plant) in 16 wild plant species exposed to vapours of ethofumesate. Error bars indicate standard deviation. Plants were fumigated for 48 h with vapours generated from 1:10 dilutions (1 g active compound in 50 ml water). Concentrations of ethofumesate in the chamber air increased to a maximum of 184 ng m^{-3} over this period.

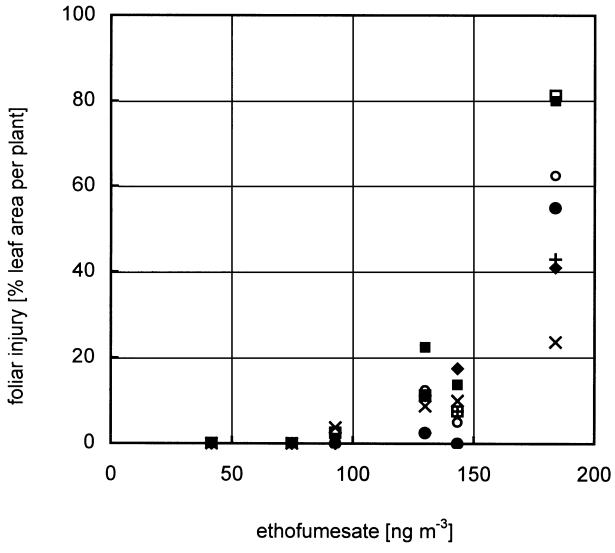

Fig. 3. Dose–response relationship between concentration of ethofumesate vapours and foliar injury in seven wild plant species. Plant species are *Hypericum perforatum* (□), *Achillea ptarmica* (■), *Lychnis flos-cuculi* (○), *Achillea millefolium* (●), *Eupatorium cannabinum* (+), *Trifolium repens* (◆) and *Agrostemma githago* (×).

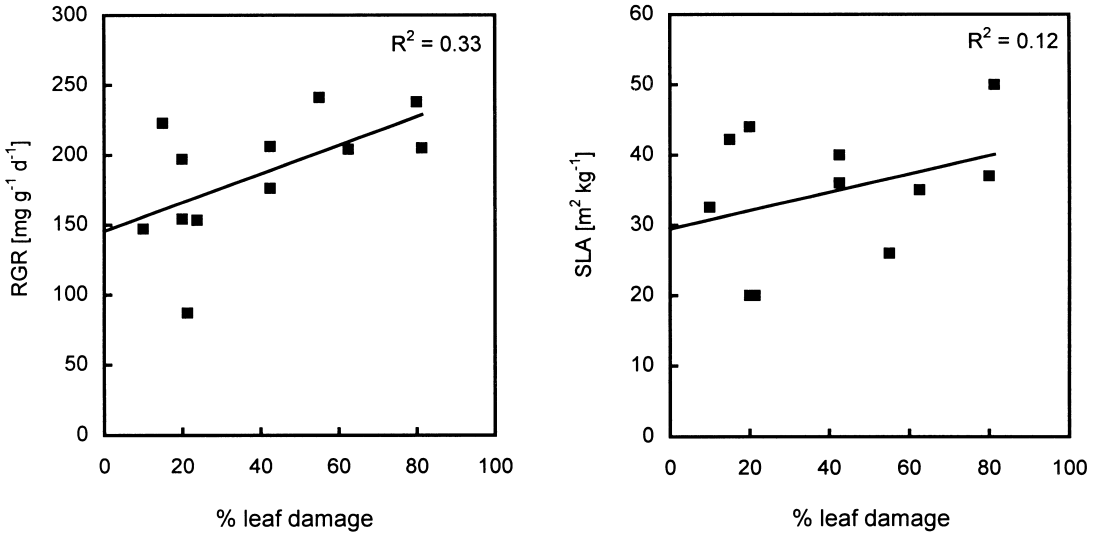

Fig. 4. Relationship between the extent of foliar injury due to ethofumesate and relative growth rate (RGR, left) and specific leaf area (SLA, right). Plants were fumigated for 48 h with vapours generated from 1:10 dilutions (1 g active compound in 50 ml water). Values for RGR and SLA are from Hunt and Cornelissen (1997) and van der Werf et al. (1998).

Kempenaar et al. (1999) did cause high mortality of four crop species, none of the plants of the wild species died in this study in which 1:10 and 1:100 dilutions were used to generate herbicide vapours.

There was a slight tendency of fast-growing species showing stronger foliar injury than the slow-growing species. Growth rates are most likely determining the effective dose of small gas molecules taken up via the stomata. The validity of this principle has been indicated before for phytotoxic effects of SO_2 and ozone (Ashenden et al., 1996, Franzaring, 2000) and such approaches may also prove useful in the ecologically oriented assessment of chemicals in the gas-phase. However, cuticular uptake of larger molecules of organic air pollutants (including pesticides) may play a greater role than stomatal uptake. Wax production rates, the surface structure of cuticles and the chemistry of cuticles will thus determine the uptake of these compounds.

Foliar symptoms due to ethofumesate vapours were not clearly related to leaf thickness (SLA) of the studied species but the symptoms were very similar in species from the same plant family. Characteristic bleaching symptoms (white flecks) due to ethofumesate were observed in the two Caryophyllaceae *Lychnis* and *Agrostemma*; the symptoms appeared first on the leaf tips and expanded into the centre of leaves a few days later. The physiological mechanism for foliar injury due to ethofumesate may be the inhibition of the synthesis of fatty acids and thus the growth limitation of the waxy cuticle, as reported by Tomlin (1994). The bleaching symptoms due to ethofumesate are very different from effects of the hormone-type growth inhibitor MCPA in *Lychnis*, which were described by Davis et al. (1994) as "flaccid, dark, crinkled leaves". However, leaf crinkling was observed in plants of *Agrostemma githago* exposed to vapours of chlorpropham. These symptoms and the observed reductions in height and growth due to chlorpropham indicate effects of the herbicide on cell division of the meristematic cells. As ethofumesate is not reported to interfere with photosynthesis, the reduced chlorophyll fluorescence determined in four species is supposed to be a secondary response to the effects of vapours of this herbicide. On the other hand, chlorpropham may affect phosphorylation (Tomlin, 1994), which could have been a direct cause of the reduced chlorophyll fluorescence determined in *Agrostemma*.

Shoot biomass and height were significantly reduced by ethofumesate only in those species that had initially responded with strong foliar injury symptoms. *Agrostemma* was the only test species showing reduced growth and height due to chlorpropham, and the "leaf crinkling" (but no leaf injury) noted a few days after the fumigation was associated with reduced growth. Species with fewer visible symptoms due to ethofumesate, however, showed no growth reductions. Plants were apparently able to recover from the herbicide stress, and the loss of photosynthetically active leaf area was compensated by the formation of new leaves.

The results of this study indicate that vapours of the pre-emergence herbicide ethofumesate may have adverse effects on established non-target wild species. NOECs for vapours of this compound causing foliar injury in sensitive wild plant species were around 75 ng m^{-3}. Vapours of the other pre-emergence herbicide, chlorpropham, did not cause foliar injury in established plants, even at concentrations of 77 μg m^{-3}. Nevertheless, it is recognised that the latter compound may affect plants in other development stages and the product information advises the user not to apply the

herbicide on plots closer than 200 m to fields with ripening tomato, cucumbers and flowering grasses. Adverse effects of chlorpropham may also be expected on the germination of seeds of non-target species and the establishment of seedlings. Negative impacts may be enhanced by its relatively long half-life values in the soil (disappearance time, DT_{50} at 15°C of 65 days; Tomlin, 1994), so that over weeks following a spraying campaign, there may be accumulation of chlorpropham in soils of a region. At higher temperatures, chlorpropham will desorb from soil particles and the compound will move into the gas phase again. The high volatility of the herbicide has been recognised by the authorities and the product must not be applied after 30 June in The Netherlands (Lohuis, 1996). Still, significant amounts of the compound may be transported from agricultural areas to non-target regions via volatilisation and re-volatilisation processes during the summer. In the past, vapours of chlorpropham have been reported to pose a serious danger to plants when adsorbed and hence desorbed by/from walls of cellars and storage facilities (Naber, 1989). The author also reports cases in which vapours from ware potatoes treated with chlorpropham to inhibit sprouting were absorbed by seed potatoes resulting in non-sprouting in the next season.

Contrary to chlorpropham, ethofumesate has relatively low soil persistence ($DT_{50} > 25$d), but it will increase strongly with rising soil organic C contents (Beulke and Malkomes, 1996). The dry gaseous or wet deposition of herbicides in remote non-target ecosystems with high contents of organic matter, e.g. forest soils, steppes, fens and peat bogs, may therefore lead to a gradual accumulation of herbicides. Eventually, concentrations of pre-emergence herbicides in the soil may reach concentrations at which germination of the most sensitive non-target species may be inhibited.

5. Conclusions

Future pesticide registration in the EU will require the evaluation of side effects on non-target plants, including both short-distance transport of droplets and long-distance transfer via vapour drift. Present data on volatilisation of herbicides from aqueous solution confirm that vapour pressure is a good predictor of ambient air concentrations. Adverse effects of herbicide vapours and NOECs, however, appeared not to be related to the volatility of the compounds. Consequently, risk assessment must not be based on chemo-physical properties alone, but also on dose–response relationships derived from fumigation experiments. Approaches should be developed in the future, in which the dose of herbicides (the product of air volume and concentration) may be related to the total leaf area of plants. Foliar symptoms proved to be a reliable indicator of adverse effects of herbicide vapours in the present study. The question remains, however, whether leaf injury due to herbicides has significant ecological impacts on the non-target vegetation. Growth reductions due to herbicides in some species may well reflect changes in competitive balances within a vegetation, but the relevant long-term changes in biodiversity cannot be predicted using such data. Phytotoxic effects of herbicide vapours occur at different levels of organisation, and flowering, seed viability and carry-over effects must be taken into account to understand the complex ecological responses. It must be concluded that it is not yet possible to scale up from a screening study to the complex situation in the field. However, fumigation studies using a variety of different plant species and vapours of different herbicides are well able to give important information on sensitive taxa and plant functional types. Studying phytotoxic effects in realistic concentration ranges of herbicides may also be assisted by effect-related monitoring programmes in the field using plant bioindicators. A suggestion for a suitable bioindicator system may be the use of clones of *Lychnis-flos cuculi* L. because this species appears to be sensitive to vapours of different herbicides.

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