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New Fungicides: Opportunities and Challenges - A Case Study with Dimethomorph

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Abstract

Dimethomorph is a novel fungicide with a high level of activity against diseases induced by certain Oomycetes, including fungal populations that are resistant to other products. In several ways, this fungicide illustrates the opportunities and challenges presented by many modern pesticides. The specific mode of action, which affects cell wall formation, is associated with a very high level of performance and low dose rates under field conditions. These low dose rates, combined with a low level of toxicity to non-target organisms present an outstanding safety profile. This same highly-specific mode of action, however, limits the spectrum of activity and suggests the need for a resistance management plan, both of which must be addressed in new product development. In addition, the biological and physiochemical properties of this, and other new products are not adequately described by the traditional classification of fungicides into "protectant" and "systemic" types. These unique profiles provide novel and useful products for disease control.

Introduction

Within the past decade several novel fungicides and classes of chemistry have been commercially introduced, with such examples of anilinopyrimidines, strobilurines, plant

defense activators, carpropamide, quinoxyfen and dimethomorph. Each has unique characteristics, strengths, and technical profiles. These novel profiles provide unique opportunities for disease management, as well as opportunities and challenges in new product development. The novel profiles of these products do not fit the traditional classification of fungicides into "protectant" and "systemic" types and often cannot be simply described. In this paper, one such fungicide, dimethomorph, is described within the context of current issues facing development of new fungicides.

Dimethomorph as an Example Fungicide

Dimethomorph [(E,Z)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-1-oxo-2-propenyl] morpholine] is a highly-effective, novel fungicide for control of many diseases induced by Oomycete fungi. The basic properties of dimethomorph were described in 1988 (Albert et. al., 1988) and the product has been commercialized in approximately fifty countries worldwide under the tradenames "ACROBAT®", "INVADER®", "SOLIDE® and "FORUM®" fungicide since 1993.

Spectrum of Activity

Like several other new fungicides, dimethomorph has a specific spectrum of activity. Dimethomorph is highly effective in controlling many important diseases induced by certain, but not all, Oomycete fungi. This includes all genera within the family Peronosporaceae (downy mildews) and the genus *Phytophthora* (late blights and root rots) (Table 1). In contrast to phenylamides, dimethomorph does not provide high levels of control of diseases induced by fungi in the genus *Pythium*.

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Table 1. Example pathogens controlled by Dimethomorph

Bremia lactucae	lettuce
Peronospora destructor	onions
Peronospora tabacina	tobacco
Pseudoperonospora cubensis	cucumbers, melons
Pseudoperonospora humuli	hops
Plasmopara viticola	vines
Phytophthora cactorum	strawberry
Phytophthora capsici	peppers, melons, cucurbits
Phytophthora cinnamoni	pineapple, ornamentals
Phytophthora cryptogea	chicory, potted plants, cut flowers
Phytophthora fragariae	strawberry
Phytophthora infestans	potato, tomato
Phytophthora nicotianae	potted plants, cut flowers
Phytophthora palmivora	potted plants, cut flowers
Phytophthora parasitica	tobacco, tomatoes
Phytophthora porri	onions, leeks

Mode of Action

The exact biochemical mode of action of dimethomorph has not been determined, but, as with many new fungicides, the mode of action is targeted towards very specific biochemical processes. Strong evidence exists that the product disrupts the biogenesis of cell walls during active growth (Kuhn *et. al.* 1991). As the cell walls of Oomycetes are unique among fungi, this may be responsible for the specific spectrum of activity.

The intrinsic level of activity of dimethomorph against plant pathogens is very high, both under laboratory and field conditions. The relative intrinsic activity of dimethomorph and other fungicides against mycelial growth of *Phytophthora infestans in vitro* is described in Table 2. This high level of activity, along with other properties, allows for dose rates that are relatively low as compared with older products (approximately 225 g a.i./ha in combinations). When combined with a high level of safety to humans and non-target organisms (Table 3), dimethomorph presents very little risk to humans and the environment (Mengle 1995).

Table 2. Intrinsic activity of Dimethomorph and other fungicides - Effects on mycelial growth of *Phytophthora infestans* - (Klinkenberg and Dehne, 1998)

Fungicide	**ED90 mycelial growth (ppm a.i.)**
Copper oxychloride	<50
Mancozeb	<50
Chlorothalonil	>50
Fentinhydroxide	>50
Fluazinam	>50
Fosetyl-Al	>50
Propamocarb	>50
Cymoxanil	<10
Metalaxyl	>50
Azoxystrobin	<1
Dimethomorph	<1

Table 3. Toxicological and ecotoxicological properties of Dimethomorph

Toxicology

Study	Result
Oral LD50(mouse)	>5000 mg/kg (male)
	3700 mg/kg (female)
Oral LD50 (rat)	4300 mg/kg (male)
	3500 mg/kg (female)
Dermal LD50 (rat)	>5000 mg/kg (male and female)
Eye irritation (rabbit)	non-irritating
Skin irritation (rabbit)	non-irritating
Inhalation 4-h LC50 (rat)	>4.2 mg/l (analytical)
Dermal sensitization (guinea pig)	non-sensitizer
Oncogenicity	Not an oncogen
Genotoxicity	Non-genotoxic
Teratogenicity	No evidence of teratogenicity
Reproduction	No effects on reproduction or growth/development of offspring.

Ecotoxicology

Organism	Result
Mallard Duck Acute LD50	>2000 mg/kg b.w.
Bobwhite Quail Acute LD50	> 2000 mg/kg b.w
Rainbow Trout 96-hour LC50	3.4 mg/l
Carp 96-hour LC50	14 mg/l
Bluegill Sunfish 96-hour LC50	>25 mg/l
Honeybee Oral LC50	>100 ug/bee
Daphnia magna 48-hour EC50	49 mg/l
Algae 96-hour EC50	>20 mg/l
Earthworm NOEL	>1000 mg/kg soil
Phytoseiulus persimilis (NOEC)	>2000 g ai/ha (WP formulation)

Systemicity

The systemicity of dimethomorph and many other new fungicides cannot be simply described. When applied to the roots, uptake of radiolabeled dimethomorph is rapid and material is translocated upward to newly formed foliage (figure 1). When applied to stems, a similar pattern is seen. Material that contacts stems after foliar application is expected to be translocated in a similar manner (figure 2). When applied to foliage, dimethomorph is absorbed rapidly and distributed through the leaf translaminarly and locally to a moderate extent, but not necessarily to newly formed foliage. As such, systemicity is best described as "penetrant with locally systemic properties" following foliar application.

These characteristics influence the optimum timing for fungicide applications. Following foliar application, material is absorbed and translocated throughout treated foliage providing rainfastness and excellent protection of foliage. As translocation is insufficient to protect newly formed leaves, foliar applications should be timed appropriately.

Effects on Stages in the Fungal Life Cycle

The effect of many modern fungicides on pathogens and pathogenic processes may also be complex, and these properties affect their overall performance and technical profiles. Dimethomorph provides and interesting example.

By affecting cell wall biogenesis, dimethomorph has been found to inhibit pathogens at all stages of their life cycles where active growth occurs (Albert *et. al.*, 1991, Albert and Heinen, 1996). Table 4 indicates the developmental stages of *P. infestans* that are affected by various "modern" Oomycete fungicides *in vitro*. Except for zoospore release, only dimethomorph effected all fungal growth stages tested. Sporulation is particularly sensitive to dimethomorph - this reduces the potential for further development of pathogen populations within the crop (Figure 4).

Unlike most of the other Oomycete fungicides described, dimethomorph is active at most stages in the fungal life cycle, providing protectant, curative and eradicant activity.

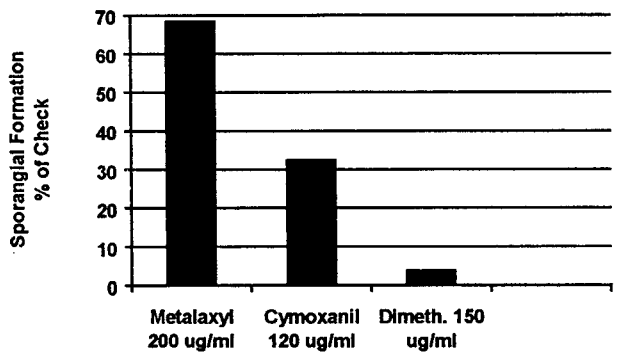
Table 4. Effects of Dimethomorph and other Oomycete fungicides on life cycle stages of *Phytophthora infestans in vitro.* (Prof. Dr. H.-W. Dehne, Univ. Bonn, pers. com.)

Compound	Mycelial Growth	Zoospore Release	Zoospore Motility	Zoospore Germination	Zoospore Germtube Growth	Sporangial Germination	Sporangial Germtube Growth
Metalaxyl*	-	-	-	-	-	-	-
Cymoxanil	+	-	-	-	-	+	+
Propamocarb	-	+	-	-	-	+	+
Phosetyl-Al	-	-	-	-	-	-	-
Fluazinam	-	+	+	+	+	+	+
Dimethomorph	+	+	-	+	+	+	+

* Metalaxyl-resistant strains used.

"-" = little or no activity, "+" = moderate to strong activity.

Figure 3. Effects of Dimethomorph on sporulation of *Phytophthora infestans in vitro*



Product Profile and Use Patterns

The sum effect of all properties; penetration, local systemicity, affects on various fungal growth stages, and sporulation is responsible for the consistent performance of dimethomorph under diverse field conditions. All actively growing stages of the fungus are affected, and the penetrant properties of the molecule allow for contact with the pathogen throughout its life cycle. In contrast, a fungicide such as fluazinam that does not penetrate leaf tissues cannot be as effective against certain growth stages. Although dimethomorph does provide curative control when applied at approximately 1-2 days after infection, optimum performance is achieved when applications are made in protectant programs. This insures that infection levels within a crop do not reach levels where disease control must rely exclusively on curative treatment.

Fungicide Resistance

As the mode of action of dimethomorph is unique, it is not cross-resistant to other fungicides, including phenylamides, and it provides excellent disease control where these populations are present.

Phenylamide resistance has become a critical problem in many areas and crops. In the Western hemisphere blue mold of tobacco (*Peronospora tabacina)* and crown rot of cucurbits (*Phytophthora capsici)* are no longer effectively controlled by phenylamides. Dimethomorph provides very high levels of disease control.

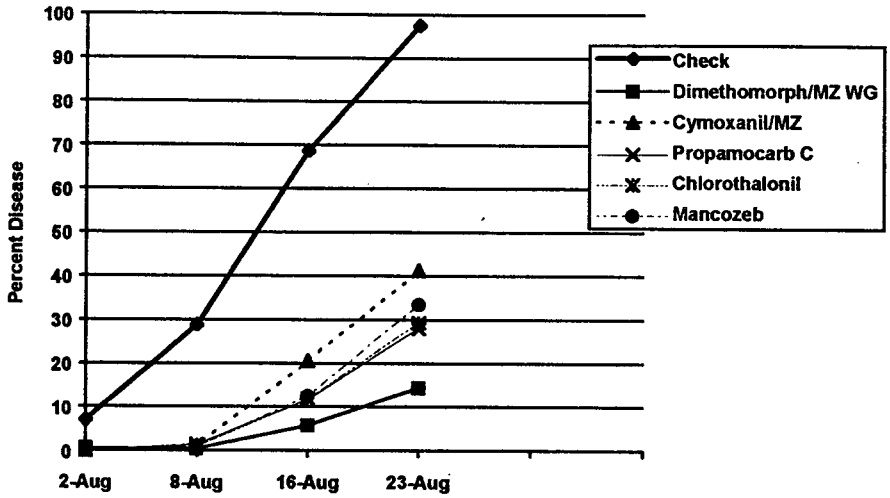
Also in the Western Hemisphere, devastating late blight epidemics have recently occurred due to the establishment of new, highly aggressive strains of *P. infestans* that are also resistant to phenylamide fungicides. Epidemics of late blight often cannot be effectively controlled with standard protectact fungicides, nor with phenylamides. The fungal strains responsible for these epidemics are effectively controlled by dimethomorph in the laboratory (table 5) and in the field (figure 5). To combat these epidemics, the US Environmental Protection Agency has granted emergency use exemptions for use of dimethomorph to control phenylamide-resistant *Phytophthora infestans, Phytophthora capsici* and *Peronospora tabacina.*

Table 5 - *In Vitro* Activity of Dimethomorph Against *Phytophthora infestans* Strains

Isolate	Mating Type	Phenylamide Sensitivity[1]	DMM Sensitivity (MIC µg/ml)[2]
FD573 (US-8)	A2	R	0.78
FD531	A2	S	1.56
FD577 (US-6)	A1	R	0.78
FD576 (US-1)	A1	R	3.13
Baseline	All	All	0.1-3.13

1. R=Resistant, S=Sensitive
2. MIC: Minimum Inhibitory Concentration

Figure 4. Control of potato late blight under field conditions. Fields were infected with aggressive, phenylamide-resistant strains of *Phytophthora infestans* (US-8) - North Dakota State Univ. - 1996



Resistance Management

As with most modern chemistries, it is assumed that there is some risk of resistance development to dimethomorph due to the specific mode of action, as well characteristics of many of the target pathogens. For dimethomorph, theoretical resistance risk assessments indicate that although the risk of resistance is predicted to be lower than that of the phenyamides, a resistance management plan is appropriate as a conservative measure.

Before commercialization resistance management programs and use guidelines were established (Mengle, 1995). The guidelines focus on effective use recommendations and integration with other crop management practices. The guidelines were also established proactively before product launch to minimize selection pressure even before commercialization and widespread use.

Use of dimethomorph in association with fungicides possessing an alternate mode of action is also an integral part of the plan, particularly for pathogens with high reproductive potential and risk of resistance. Although there is no convincing data to favor the use of coformulations over rotations, coformulations of dimethomorph, and appropriate partner fungicides have been a major component of this plan.

Coformulations can be considered a more desirable option as compared to tank mixing in that they provide a convenience to the grower, and that it is more likely that the two products will be applied together, and in the appropriate ratio. It is recognized that due to regulatory and other constraints, coformulations may not be universally available for all markets and uses. In such cases, alternations or tank mixes with products possessing alternatives modes of action are recommended for high-risk pathogens.

Formulations

Despite the expense of development and registration of each new formulation (often exceeding 1.0M$ US) a range of dimethomorph coformulations have been developed, or are in the process of registration for use on high-risk pathogens, such as *Plasmopara viticola* and *P. infestans.* This range of coformulation products has been developed, in part to address market needs, but also to address regulatory issues in specific countries, where certain partner fungicides are viewed as unacceptable.

Most formulations are being developed as wettable granule (WG) or liquid formulations for convenience to the user, as well as to address increasing safety concerns and regulatory pressure on wettable powder (WP) formulations. Package disposal issues are also becoming more important in many countries, favoring the development of WG formulations.

Discussion - New Actives and Product Development

The discovery of novel target sites are a primary objective of most research programs. These target sites provide products with ever-increasing levels of activity and margins of safety, to provide unique solutions to crop protection challenges, and to address problems of pesticide resistance.

Mechanism of Action

The biochemical modes of action of many modern fungicides tend to be more specific than traditional fungicides, such as dithiocarbamates, pthalamides, chlorothalonil, and copper containing products. As such, the new fungicides are more targeted than those of the past. This can be responsible for more narrow spectra of activity, but also for their excellent crop safety. Dimethomorph provides an excellent example of the benefits and challenges associated with products having novel and highly-specific mechanisms of action. The mode of action appears to be targeted to biochemical process that are more sensitive in the pest (if not specific to it) than to other organisms. As such, the margin of safety to humans, non-target organisms and the environment is generally very high.

Modern products are often highly active against these processes - the result is that very low dose rates are required to achieve pest control. This dramatically reduces the amount of active ingredient required for effective disease control, and consequently further reduces the potential impact against non-target organisms. Modern fungicides may also require less frequent applications, resulting in further reductions in environmental impact.

As global standards for safety to humans and the environment continue to increase, such excellent safety profiles will continue to be expected in new chemistries.

Spectrum of Activity

A challenge for many new pesticides is the economic justification for product development. Once discovered and characterized, registration and development of a new active ingredient easily requires expenditures of about 50M$ US to meet modern registration standards, to obtain global registrations and to develop the high-quality formulations expected in a competitive marketplace. The decision to enter a new active ingredient into development and commercialization requires an increasingly rigorous analysis of it's commercial potential.

The spectrum of activity is certainly not the only factor which enters into this decision, but modern compounds with relatively narrow spectra of activity present more challenges in this regard. Therefore, the many benefits associated with a specific mode of action, including high levels of activity, the ability to control resistant strains of pathogens, health and environmental safety, and unique properties are considered.

Therefore, a critical factor in determining the commercial potential of a new pesticide is not strictly it's spectrum of activity, but also the need for new products in specific markets and how well a new active ingredient will address these needs. A product with a relatively narrow spectrum of activity, such as dimethomorph may provides unique solutions to important disease control challenges.

Fungicide Resistance

Modern crop protection agents with highly-specific modes of action are generally regarded as having higher risk for fungicide resistance than traditional protectant products with more general modes of action. The development of fungicide resistance presents new opportunities for fungicides that can control resistant strains of pathogens, but also a challenge and risk for these new active ingredients.

The importance of resistance management for new actives is generally recognized, as exemplified by the European Union Registration Directive. The applicant for a new fungicide must provide an assessment of the risk of fungicide resistance. If the risk is judged to be significant, a resistance management plan must be provided. Considering the increasing costs of development and registration, fungicide resistance and it's management must be considered in determining if a new active ingredient should be considered for development and how it should be used.

Once a decision to fully develop a new product is made, proactive implementation of resistance management strategies can be an important component of new product commercialization. Development and implementation of such resistance management strategies is a challenge as the quantification of resistance risk potential is not an exact science. This may complicate the establishment and implementation of resistance management strategies - particularly as these strategies may appear to be inconsistent with commercial goals, at least in the short term.

Many resistance management programs rely on mixtures or alternations with other products, particularly products that have a low risk for resistance. In general, coformulations and tank-mix recommendations are typically based on a combination of an "at risk" fungicide with a traditional, multi-site fungicide with a low risk of resistance (e.g. dithiocarbamates, copper fungicides, etc.) rather than with two "at risk" fungicides with different modes of action. This is to avoid the possibility of multiple resistance. At this time, there is very little data available on value of such mixtures as compared with mixtures containing "low-risk" fungicides.

Therefore, maintaining a diversity of products for use is an important aspect of resistance management, and will become a challenge, considering increased emphasis on re-registration of older chemistries.

Product Characteristics

Fungicides have frequently been characterized as either "protectants" or "systemics". Rather than restricting these terms to their exact definitions, other characteristics are frequently and incorrectly associated with these terms (table 6). This may result in confusion when applied to a specific product.

Although convenient, use of the above categories may lead to a misunderstanding of the properties and benefits of new chemistries and often does not accurately reflect their unique properties.

Table 6 - Characteristics associated with fungicide terminology

"Protectant Fungicide"	"Systemic Fungicide"
Contact or protectant activity only	Protectant and curative activity
Multi-site mode of action	Single-site mode of action
Broad spectrum	Narrow spectrum
Less prone to resistance	Higher risk of resistance
More toxic to non-targets	Less toxic to non-targets
Higher dose rates	Lower dose rates
Shorter spray intervals	Longer spray intervals

For example, dimethomorph has often been described as a "systemic fungicide", which is technically correct. However, the product is sometimes associated incorrectly with all of the properties attributed to this term, such as curative activity and the ability to completely control disease on new growth. Optimum performance, however, is obtained by use in protectant, rather than curative programs, although the product does possess curative properties. In addition, the degree of systemicity is best described as a "penetrant" or "locally systemic" following foliar application. The association with the term "systemic" has in some cases led farmers to believe that new plant growth can be adequately protected following foliar applications.

Categorization as a "protectant fungicide" is also incorrect and misleading as the product will outperform products that do not possess the same degree of systemicity, protectant, curative

or anti-sporulant activity. In summary, dimethomorph, as is the case with several other new fungicides does not fit either of these traditional models, and is best categorized in terms of it's unique and specific properties.

As new fungicides with unique profiles, such as dimethomorph, are discovered and developed these terms become less accurate and less useful. A new challenge for the development and commercialization of such products is therefore the accurate communication of the unique characteristics and optimum use strategies of modern fungicide chemistries.

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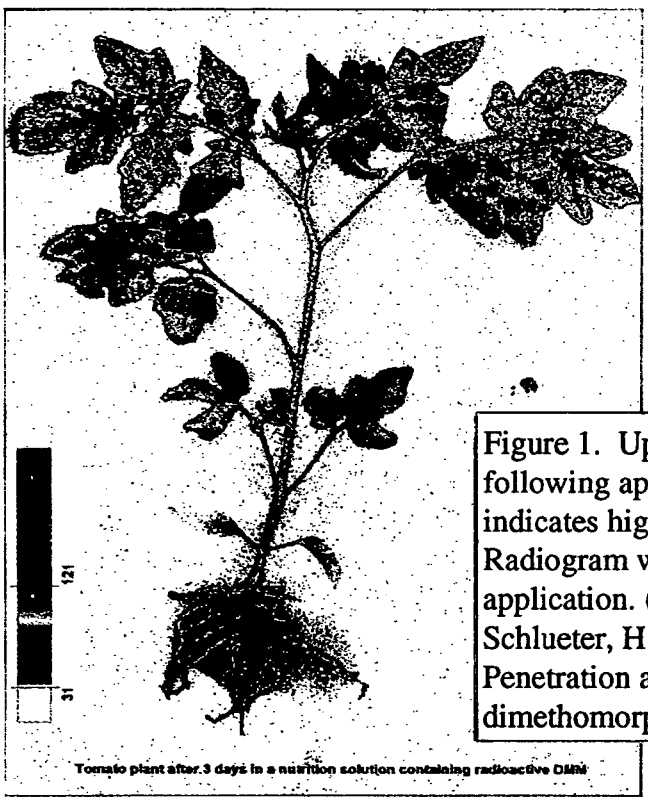

Figure 1. Uptake of radiolabeled dimethomorph following application to tomato roots. Red indicates highest concentrations of radioactivity. Radiogram was taken three days after application. (Data from Albert, G., Eichler, D., Schlueter, H. (manuscript in preparation) Penetration and translocation of the fungicide dimethomorph in plants)

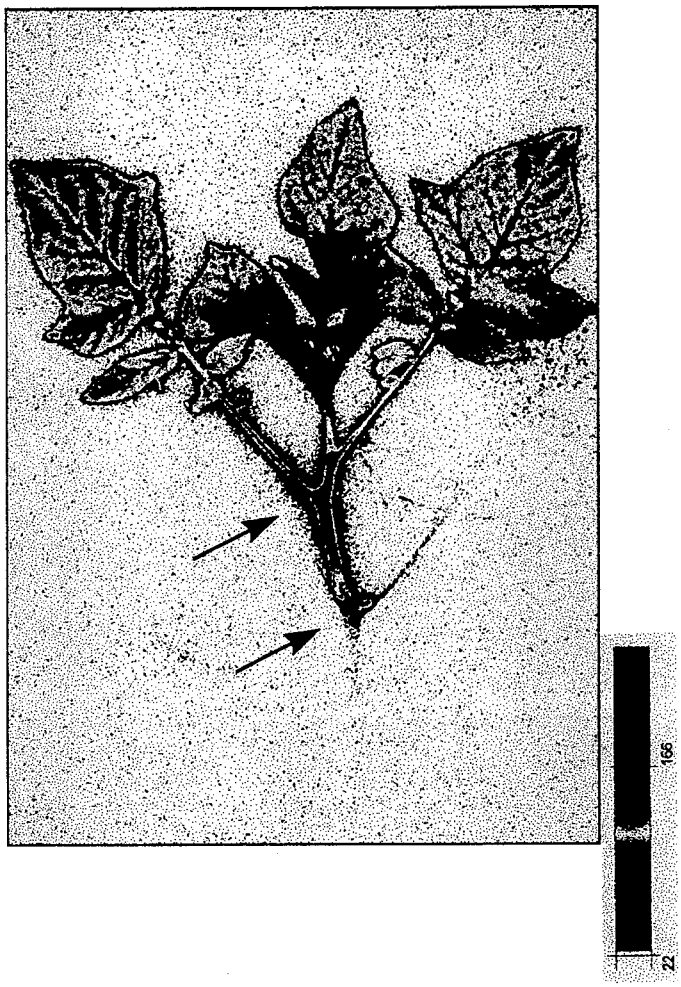

Figure 2. Uptake of radiolabeled dimethomorph following application to a potato stem.
Red indicates highest concentrations of radioactivity.
Radiogram taken 1.5 days following application.
Arrows indicate region of application.
(Data from Albert, G., Eichler, D., Schlueter, H. (manuscript in preparation) Penetration and translocation of the fungicide dimethomorph in plants)