

Session 4

Seed Treatment for Non-Graminaceous Crops

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SEED TREATMENT USAGE ON PEAS AND BEANS IN THE UK

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ABSTRACT

A range of seed treatments are used in the UK to protect against several soil- and seed-borne fungal pathogens. Almost all pea seed, but only a small proportion of field bean seed is treated. Protection against damping-off, *Ascochyta* spp. and *Peronospora viciae* is achieved in peas using either single or multi-purpose products, depending on the health of the seed and the varietal susceptibility to disease. The necessity for such a range of protection in winter and spring field beans is discussed. Only limited success has been achieved in controlling root diseases in both crops and no insecticidal seed treatments are available for peas and beans to control seedling pests.

INTRODUCTION

The use of fungicidal seed treatment in peas (*Pisum sativum*) has been well established for many years. The crop is grown either for human consumption, for animal feed compounding or for seed. In 1992, vining peas, harvested green for freezing or canning occupied 44,900 ha in the UK whilst combining peas, harvested dry, occupied 78,600 ha (MAFF, 1993). Up to 75% of vining pea seed is imported, mainly from the USA, already treated with seed protectants, whilst the UK is virtually self-sufficient for combining pea seed and therefore, it is all treated in the UK.

Seed treatments are used less extensively on field beans (*Vicia faba*). The crop is either autumn or spring planted and most of the produce is used for compounding with a small tonnage either exported or used for human consumption. The area under production in the UK in 1992 was 129,100 ha, with virtually the entire seed requirement being produced in this country.

Peas are more susceptible to seed-bed losses as a result of soil-borne fungal pathogens and the crop is also susceptible to seed-borne fungi. In the UK, seed treatments are used extensively for protection against a range of fungal pathogens.

The bean crop has expanded rapidly in recent years as a result of changes in the EEC area payment schemes. The range of varieties, particularly of spring sown beans, has been enlarged with new introductions which include types with low tannin in the seed. Hitherto, varieties were of the coloured flowered type with high seed tannin levels. Recent work has sought to evaluate any difference in susceptibility of the low tannin seeds to soil-borne pathogens.

This paper outlines the major uses of seed treatments in the pea and bean crops and discusses the possibilities for future seed and seedling protection.

SEED BED LOSSES

Pea seed, especially vining pea varieties, are particularly susceptible to pre-emergence losses caused by damping-off. The early-maturing varieties are often drilled early in the spring when soil temperatures are low and seedbeds are relatively wet (Gane & Biddle, 1978). Leakage of seed exudates under these conditions attracts *Pythium* spp. which colonise the cotyledons and become pathogenic. Dithiocarbamates are, therefore, used extensively to protect the newly germinating peas from infection.

Beans appear less susceptible to such losses. Coloured-flowered varieties often contain tannins in the seed and as such appear to be more resistant to some of the soil-borne fungal pathogens. Some new white-flowered types, however, seemed to be susceptible to fungal attack and Kantar, F., Hebblethwaite, P.D. & Pilbeam, C.J. (unpublished) found that fungicidal seed protectants were of value. With the introduction of commercially acceptable varieties of low-tannin beans, the value of seed treatments was again examined. In a series of field trials carried out by the author, between 1991 and 1993, coloured and white-flowered varieties of autumn-planted-winter and spring-planted field beans were treated with a range of seed treatments which included thiram, alone or in combination with thiabendazole, and metalaxyl. In the three years of experiments carried out at two sites, Thornhaugh and Boxworth, Cambridgeshire, there were no significant differences in plant emergence or in yield between any of the treatments or with untreated seed. The results therefore, cast doubt on the benefits of routinely treating field bean seed where protection solely from soil-borne damping-off diseases is required.

SEED-BORNE DISEASES

Ascochyta spp. remain the most common and important fungal pathogens in both peas and beans. In peas, leaf and pod spot caused by *Ascochyta pisi* is now uncommon in the UK, but it has been replaced in importance by a related disease caused by *Mycosphaerella pinodes*. Unfortunately, for the purposes of control by seed treatments, *M. pinodes*, unlike *A. pisi*, produces soil-borne chlamydospores which can infect crops later in the season, particularly during wet summers. As a seed-borne disease, *M. pinodes* causes a rot in the hypocotyl region of the pea seedling resulting in seedling death, or a more general leaf and pod spot disease.

Seed produced in the UK is often infected to a significant degree by *M. pinodes* and the proportion of seedlots containing infection is relatively high in many years (Biddle, 1986). Control of *A. pisi* and *M. pinodes* can be effected by MBC fungicides including thiabendazole (Biddle, 1981) and it is current practice to treat seed where infection is between 5% and 35%. Thiabendazole treatment for this purpose is complemented with the addition of thiram for protection against damping-off. Recent work in France has suggested that strains of *M. pinodes* may be resistant to

thiabendazole. There have been no such reports in the UK, however several other fungicides are currently being evaluated as alternatives (PGRO, 1991 and 1992).

In field beans, *Ascochyta fabae* is the most serious fungal pathogen. Winter sown beans are more likely to suffer from a seed-borne infection as the disease can spread more rapidly during the wet conditions which can occur during the late winter and early spring. Following the 1992 harvest season, winter beans were more seriously infected by *A. fabae* than for many years. The results of tests carried out on 451 seed samples by PGRO in the autumn of 1992 are shown in Table 1.

TABLE 1. *Ascochyta fabae* levels in winter bean seed lots tested by PGRO in 1992

Level of seed infection (%)	All seedlots (%)	Farm-saved (%)	Certified at C ₂ level (%)
< 1	69	71	67
1-5	23	25	21
5-10	5	3	7
> 10	3	1	4

Seed-borne infection of *A. fabae* is not so effectively controlled by thiabendazole seed treatment alone. Reports have shown between 60-80% reduction in disease levels (Jellis *et al*, 1988). Because field infection is also difficult to control with foliar sprays, and because infection can be severe in wet seasons, standards have been in place in the UK Field Bean Certification Scheme whereby the maximum seed infection allowed is 1% in the C₂ generation. Earlier generations have much higher standards. It has also been demonstrated that the sexual form of *A. fabae* (*Didymella fabae*) can develop on infected crop debris and air-borne spores may be dispersed by wind for long distances (Jellis & Punithalingam, 1991). It is, therefore, very important that the initial seed health standards be maintained. It is currently recommended by PGRO that uncertified bean seed should be treated if the level of *A. fabae* is between 1% and 3%. Seed with levels higher than this should be discarded (PGRO, 1993).

SOIL-BORNE DISEASES

Both peas and field beans can be affected by downy mildew caused by *Peronospora viciae*. At present, however, there appear to be different strains of *P. viciae* infecting the two crops (J.E. Thomas, 1993, personal communication). The fungus survives in the soil for many years and seedlings can then become infected shortly after germination. Infected pea seedlings emerge as pale, stunted plants on which the fungus produces air-borne spores. In beans, particularly spring varieties, the disease has become more common in recent years. Early seedling symptoms are not seen as commonly as with peas, but during the flowering stage, leaves can develop irregularly shaped, pale lesions which develop into larger areas

resulting in defoliation. The growing points may also become systemically infected and further growth and pod set is reduced. There is a range of susceptibility exhibited by both peas and field beans; the current status of combining peas and spring beans is published in the NIAB Recommended list of field peas and field beans (NIAB, 1993). Vining pea varieties are tested by PGRO and Table 2 summarises the varietal susceptibility ratings obtained from the 1992 and 1993 tests for some of the newer varieties.

TABLE 2. Susceptibility of vining pea varieties to downy mildew, PGRO 1992-93.

Rating:	1	3	5	6	7	9
Avola (Standard)		Caty	Deltafon	CMG282	Ambassador	Minado
CMG 264 F		FR774	Sublima	Co400	Bastion	Solo
		Polo		Cobalt	Lambado	
		Rexado		Lynx		
		Sancho				
		Winner				

The highest ratings relate to the highest level of varietal resistance.

Seed treatment has been shown to be very effective in protecting newly germinated pea seedlings from infection by the soil-borne source of inoculum (Miller & de Whalley, 1981; Vulsteke & Meeus, 1985). Metalaxyl, in combination with thiabendazole and thiram has been in general use in the UK for several years, providing control of downy mildew and seed-borne *Ascochyta* spp. (Salter & Smith, 1986). A recent introduction to the UK has been oxadixyl, again in combination with thiabendazole and thiram. However, whilst either treatment prevents primary infection of peas, secondary infection from air-borne spores introduced from a neighbouring source is not necessarily reduced. Although there is some difference in varietal susceptibility between vining pea varieties, all seed used in the UK is treated with either metalaxyl- or oxadixyl-based products. In combining peas, there are more varieties which show a high level of field resistance and therefore only those which are rated by NIAB as 6 or below are treated routinely.

In spring beans, foliar spraying of fungicides is effective in controlling downy mildew. However, recent work has indicated the potential for the use of systemic fungicides as seed treatments. This form of control may, in the long-term, be a more cost-effective option than the current practice of crop spraying.

FOOT ROT DISEASES OF PEAS AND BEANS

Several soil-borne fungi are capable of infecting the roots or stem bases of peas and beans either as individuals or in concert. Peas are susceptible to *Fusarium solani* f. sp. *pisi* and *Phoma medicaginis* var.

pinodella which together cause a foot rot, especially where the crop has been grown frequently in the past (Biddle, 1983). Field beans are also susceptible to such infection, but in the last two years, 1992 and 1993, there has been a notable increase in stem base infection of spring beans attributed to *Fusarium culmorum* and *F. solani* (PGRO, 1992).

Several studies have shown the effects of seed treatment in reducing the pea disease complex in laboratory or glasshouse experiments (Gravanis, 1986; Bradshaw-Smith, 1991), but only limited reduction has been achieved in the field (Salter & Smith, 1986). Biological control agents coated onto seeds have included *Pythium oligandrum* and this also showed some effects under controlled conditions, but field tests did not confirm the earlier findings (Bradshaw-Smith *et al*, 1991).

In field beans, there appear to be varietal differences in susceptibility to the *F. culmorum*/*F. solani* complex (J.E. Thomas, 1992, personal communication), but in one field trial carried out at Thornhaugh in 1992, seed treatment mixtures applied to a susceptible variety failed to reduce infection significantly (Table 3).

TABLE 3. Effect of seed treatments on stem disease in spring beans, cv. Caspar, 1992

Treatment	seedling emergence (m ⁻²)	% infection by stem rot	yield (t/ha)
untreated	31.4	13.0	4.39
thiram	21.1	5.3	5.75
thiram + thiabendazole	29.6	12.3	4.94
metalaxyl, thiabendazole and thiram	31.8	12.7	5.15
SED	2.8 (nsd)	4.1 (nsd)	0.4 (nsd)
CV%	11.7	53.8	12.8

SEEDLING PESTS

Both peas and beans can be attacked by the pea and bean weevil (*Sitona lineatus*) and field thrip (*Thrips angusticeps*). Experimental work has shown that incorporated insecticide granules are more effective than sprays in reducing damage and increasing yields (King, 1981; Biddle, 1985) but the addition of an insecticide to the seed treatment has produced even better results (Baughan *et al*, 1985; Salter & Smith, 1986). However, the insecticides used experimentally had high avian toxicity and thus far, in the UK, there are no products Approved for use on peas or beans.

DISCUSSION

The use of seed treatments, either as single-purpose or multi-purpose treatments for seed and soil-borne pathogens is well established for peas

in the UK. The most common seed-borne disease, *M. pinodes*, occurs frequently during wet seasons, but is effectively controlled at present by the fungicides available. If resistance develops in the UK, there will be an urgent need for alternative materials. Downy mildew is also common as a soil-borne pathogen and, whilst the introduction of new varieties continues, only a small number show stable varietal tolerance and systemic acyl-analines are required for all vining pea and many combining pea varieties.

Little progress has been made in reducing root pathogens in both peas and beans and the seed treatments used currently are of little benefit in the field as they do not protect the plants beyond the seedling stage.

In beans, the requirement for seed treatment appears not to be so important. Seedling establishment, even after winter bean seed has been ploughed-in, does not appear to be affected by seed treatment, nor do the newly introduced tannin-free varieties appear to be more susceptible to seed bed losses. However, the improvement in control of *A. fabae* in the seed is a desirable goal and the further development of fungicides for the control of downy mildew in spring beans is also worthy of pursuit, as is the broader subject area of the use of environmentally more benign insecticidal seed treatments.

REFERENCES

- Baughan, P.; Biddle, A.J.; Blackett, J.A.; Toms, A.M. (1985) Using the seed as a chemical carrier. *Symposium on application and biology, BCPC Monograph No. 39*, Croydon: BCPC Publications.
- Biddle, A.J. (1981) Pea seed treatments to control *Ascochyta*. *Tests of Agrochemicals and Cultivars, Annals of Applied Biology* 97, Supplement, No. 2, pp 34-35.
- Biddle, A.J. (1983) The foot rot complex and its effect on vining pea yield. *Proceedings 10th International Congress of Plant Protection*, 1, 117.
- Biddle, A.J. (1985) Pea pests - yield, quality and control practices in the UK. In: *The Pea Crop - a Basis for Improvement*, P.D. Hebblethwaite, M.C. Heath and T.C.K. Dawkins (Eds), London, Butterworth.
- Biddle, A.J. (1986) Seed treatments for peas - a review. *Aspects of Applied Biology* 12, *Crop Protection in vegetables*, pp. 129-134.
- Bradshaw-Smith, R. (1991) Chemical and Biological Control of Fungal Foot Rot Pathogens of *Pisum sativum* L. PhD Thesis, Manchester Polytechnic. pp 305.
- Bradshaw-Smith, R.; Craig, G.D.; Biddle, A.J. (1991) Glasshouse and field studies using *Pythium oligandrum* to control fungal foot rot pathogens of peas. *Aspects of Applied Biology*, 27, *Production and protection of legumes*, 347-350.
- Gane, A.J.; Biddle, A.J. (1978) Pea establishment problems - a review. *Acta Horticulturae* 72, 121-124.
- Gravanis, F.T. (1986) A study of the *Fusarium* foot and root rot of peas and an evaluation of certain chemicals for its control. PhD Thesis, University of Manchester. pp 228.
- Jellis, G.J.; Bolton, N.J.E.; Clarke, M.H.E. (1988) Control of *Ascochyta fabae* on faba beans. *Brighton Crop Protection Conference - Pests and diseases*, 895-900.

- Jellis, G.J.; Punithalingam, E. (1991) Discovery of *Didymella fabae* sp nov, the teleomorph of *Ascochyta fabae* on faba bean straw. *Plant Pathology* 40, 150-157.
- King, J.M. (1981) Experiments for the control of peas and bean weevil (*Sitona lineatus*) in peas. 1981 *British Crop Protection Conference - Pests and Diseases*, 1, 327-331.
- Ministry of Agriculture, Fisheries and Food (1993) *Agricultural and Horticultural Census: 1 June 1992, United Kingdom, Final Results*.
- Miller, M.W.; de Whalley, C.V. (1981) The use of metalaxyl seed treatments to control downy mildew. 1981 *British Crop Protection Conference - Pests and Diseases*, 1, 342-348.
- National Institute of Agricultural Botany (1993) Recommended varieties of field peas and field beans 1993. *Farmers Leaflet No. 10*. NIAB, Cambridge, UK, 18 pp.
- Processors and Growers Research Organisation (1991, 1992) *Annual Reports of the Processors and Growers Research Organisation*, 1991 and 1992. Peterborough, UK.
- Processors and Growers Research Organisation (1993) Notes on growing field beans. *Advisory Leaflet*, Processors and Growers Research Organisation, Peterborough, UK. 11 pp.
- Salter, W.J.; Smith J.M. (1986) Peas - control of establishment of pests and diseases using metalaxyl based seed coatings. *Aspects of Applied Biology*, 12, *Crop Protection in Vegetables*, 135-148.
- Thomas, J.E. (1993) Personal Communication. National Institute of Agricultural Botany.
- Vulsteke, G.; Meeus, P. (1985) Control of *Peronospora viciae* in peas. *Medelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* 50, 1205-1215.

CONTROL OF PESTS AND DISEASES IN SUGAR BEET BY SEED TREATMENTS

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ABSTRACT

The current and future use of fungicides and insecticides as seed treatments to control seedling diseases and pests of sugar beet in the UK is reviewed. Thiram and hymexazol have become the standard treatments for seed- and soil-borne pathogens throughout Europe, and these chemicals continue to give good control in the UK in comparison to candidate alternatives. Two new insecticides, tefluthrin and imidacloprid are now available to UK growers as more effective alternatives to the standard methiocarb. Establishment of sugar beet in crops attacked by arthropod soil pests was as good with both insecticides as with carbamate granular insecticides. Imidacloprid also gives excellent control of foliar pests, particularly virus-carrying aphids. The benefits to the environment through introduction of these seed treatments is discussed.

INTRODUCTION

Control of pests and diseases attacking sugar beet is essential under present-day husbandry which leaves little scope for plant losses, particularly at the vulnerable seedling stage. In the UK, this has been achieved by the extensive use of pesticides; until 1992 all sugar-beet seed was treated with two fungicides and one insecticide, and additional insecticides were applied to the soil at or around drilling time to approximately 60% of crops (Asher & Payne, 1989; Winder *et al.*, 1993).

This paper examines the progress made with sugar-beet seed treatments since 1988 (Dewar *et al.*, 1988), describing the continuing search for alternative fungicides to what are now the standard thiram and hymexazol treatments, and the introduction of two new insecticides.

FUNGICIDES

Control of seed-borne *Phoma betae*

The use of the thiram steep process prior to pelleting has largely eliminated disease caused by *Phoma betae* from commercial seed sown in the UK. The additional benefit of the prolonged steep which physiologically "advances" seed, giving more rapid emergence and improved crop establishment, has been documented elsewhere (Durrant *et al.*, 1988). Despite this progress, the search has continued for possible alternative fungicides to thiram for use in the steep process, particularly for environmental reasons.

Table 1 shows the results of three trials on different sites in 1992, examining the effect of several different fungicides on the emergence of seedlings from *Phoma*-infected seed. Processed and graded seed with 67% *P. betae* infection was steeped for 12 h at 25°C in either a 0.2% aqueous suspension of thiram or a 0.1% aqueous solution of guazatine (Rappor, Dow-Elanco), iprodione (Rovral, Rhône-Poulenc), tolclophos-methyl (Rizolex, Hoechst-

TABLE 1. Percentage emergence of seedlings from *Phoma*-infected sugar-beet seed at three trial sites in 1992 following treatment with fungicides in a 12 h steep.

Treatment	Site		
	Higham, Suffolk ^a	Potterhanworth ^b	Gayton, Norfolk ^c
Water	75.7	75.1	11.2
Thiram	89.6	88.3	42.1
Guazatine	84.2	82.3	48.3
Iprodione	65.1	53.4	5.0
Tolclophos-methyl	61.3	53.9	3.7
Fenpiclonil	62.5	46.7	6.0
5% L.S.D.	6.1	13.1	4.5

^aSown 10.4.92, counted 13.5.92; ^bSown 9.4.92, counted 22.5.92; ^cSown 29.9.92, counted 22.10.92

Schering) or fenpiclonil (Beret, Ciba-Geigy). A water steep was included as the control. After air-drying at room temperature, the seed was pelleted by Germain's (UK) Ltd. with the standard EB3 coating but omitting all pesticides. Seed was machine drilled at three sites. At each site the six treatments were replicated six times in a randomised block or latin square design, using 12 m long single-row plots of 250 seed at ca 4.5 cm spacing.

Seedling emergence was generally high at the two sites sown in the spring but much lower in the autumn-sown trial (c). The performance of all treatments was compared with the water-steep treatment (without fungicides) which is known from previous experience (P.A. Payne, unpublished) to give some control of *P. betae*. Emergence was consistently better with both thiram and guazatine than with the water control whereas the other chemicals were significantly less effective, especially at Gayton. Previous work in controlled environments (P.A. Payne, unpublished) had suggested that the 0.1% concentration adopted for these fungicides (which are, in general, more soluble than thiram) was optimal for control and not phytotoxic. A range of concentrations of the active chemicals should now be studied under field conditions.

A recent survey (Dewar & Asher, in press) of seed treatments in use on sugar-beet in Europe has highlighted the popularity of thiram applied as a dust or aqueous suspension to the seed and/or incorporated in the pelleting material. Over 1.8 million ha were sown with seed treated with this fungicide in 1992.

Control of soil-borne diseases

The most important soil-borne diseases at the seedling stage in the UK are those caused by *Pythium* spp. and *Aphanomyces cochlioides* (Payne *et al.*, 1994). Since 1988, hymexazol (Tachigaren, Sankyo, Japan) has been applied in the pellet to all seed used commercially in the UK, at a rate of 10.5 g per kg seed, to control these pathogens (Payne & Williams, 1990). However, *Pythium* spp. are also very effectively controlled by a combination of the thiram steep treatment and the pelleting process. Table 2 shows the results of applying different combinations of these treatments to rubbed and graded seed that was subsequently hand-sown in soil naturally infested with *Pythium* spp. Seed which had been pelleted but which had not received any fungicide treatment, either prior to or during the pelleting process, were attacked less than the untreated control. Steeping seed in thiram suspension, but without subsequent

TABLE 2. Effect of the thiram steep and the EB3 pellet on the incidence of infection by *Pythium* spp. in naturally infested field soil at 20°C.

Thiram steep	Treatment		% infected seedlings
		EB3 Pellet ^a	
— ^b		—	50.1
— ^b		+	41.2
+		—	19.8
+		+	3.9

^a without fungicides; ^b water steep only; 5% L.S.D. = 8.7

pelleting, markedly reduced disease incidence. The combination of both treatments virtually eliminated the disease. *Pythium* spp. are known to invade seed during the early stages of germination, leading primarily to pre-emergence seedling losses. Clearly, protecting the seed with a physical barrier such as the pellet, and impregnating it with a fungicidal chemical, both contributed to the high level of disease control that was achieved.

The additional benefit of incorporating hymexazol in the pellet is the control of *A. cochlioides*, against which thiram and metalaxyl-based fungicides are ineffective (Bruin & Edgington, 1983). A recent survey (Dewar & Asher, in press) shows that hymexazol was used on sugar-beet seed on over 2.4 million ha in Europe in 1992 at rates varying from 5.6 - 28.0 g per kg seed, depending on the disease pressure in the country or region. The prevalence of *A. cochlioides* and the lack of viable alternative chemicals for its control, suggest that hymexazol will remain in widespread use on sugar beet seed for some time.

INSECTICIDES

Control of seedling pests in the soil

Since 1987, when methiocarb was the only insecticide applied to sugar beet seed pellets in the UK (Dewar *et al.*, 1988), two new active ingredients have been used in insecticides now available to growers. The first to be introduced in 1992, was tefluthrin (Force ST; Zeneca Crop Protection). Tefluthrin is a soil-stable synthetic pyrethroid with a high level of activity against soil insect pests (Jutsum *et al.*, 1986; McDonald *et al.*, 1986). It is applied to the surface of sugar beet pellets at 10 g AI per unit (1 unit = 100,000 seeds) in a micro-encapsulated formulation which allows slow release of the active ingredient, thus prolonging persistence (Marrs & Gordon, 1988). It has low solubility, but its vapour activity forms a 'seedling protection zone' around the young roots and hypocotyl, which is effective even in dry soil conditions.

In trials from 1986-1989, on sites where soil pests such as springtails (predominantly *Onychiurus* spp.), millepedes (predominantly *Blaniulus guttulatus* and *Brachydesmus superus*) and symphylids (*Scutigera immaculata*) had been reported to cause problems in previous sugar beet crops, tefluthrin gave the best establishment in comparison to carbosulfan and furathiocarb (Dewar *et al.*, 1988; Winder, 1990). Large-scale grower trials carried out by British Sugar plc Agricultural Development staff from 1989-91 confirmed that tefluthrin performed well over a wide range of soil types giving comparable establishment to that achieved by carbamate granules such as aldicarb and carbofuran (Cook *et al.*, 1991). Similar benefits were achieved in two series of collaborative trials across Europe organised by the members of the Pests and

Diseases Group of the Institut International de Recherches Betteravieres (IIRB) in 1987 and 1988 (Dewar, 1989), and by ICI plc and SOPRA in the UK and France (Moran *et al.*, 1988).

Tefluthrin was first introduced commercially in France in 1988 and is now also available in the Netherlands, Belgium and Germany. In 1992 in Europe, it was used on a total of 160,000 ha (Dewar & Asher, in press) mostly applied at 12 g AI/unit, but as low as 4 g AI/unit in France. More recent trials conducted in France, and by members of the IIRB Pests and Diseases group, have shown that lower rates performed as well as the higher (Moran *et al.*, 1988; Muchembled, 1991; Dewar, 1992a).

This seed treatment is cheaper than the alternative granular insecticides (approximately half the price per ha) and much less active ingredient is applied to the environment. In Britain at average seed sowing rates (1.15 units/ha) only 11.5 g of active ingredient is applied per hectare compared to between 510 and 760 g AI for the various carbamates available - a substantial (at least 97%) reduction. This reduction, coupled with tefluthrin's non-soluble relative stability (Marrs & Gordon, 1988), results in very few adverse effects on non-target organisms, especially compared to sprays of the broad spectrum α -HCH (lindane) (Dewar *et al.*, 1990; Coulson *et al.*, 1990).

However, because tefluthrin is non-systemic, it has little or no effect on arthropod pests which attack young seedlings outside the soil environment. Nor does it control either beet cyst nematode (*Heterodera schachtii*) or the free-living nematodes (*Longidorus* and *Trichodorus* spp.) which cause Docking disorder (Cook *et al.*, 1991). Therefore growers with these pest problems must either continue to rely on granules or sprays, or consider the other new seed treatment, imidacloprid, at least for foliar pests.

Control of soil and foliar pests of seedlings

Imidacloprid (Gaucho; Bayer plc) is a member of a novel group of insecticides, the nitroguanidines, and has a different mode of action to that of organophosphorus-, carbamate- and pyrethroid-insecticides, based on an interaction with the nicotinic acetyl-choline receptors on the post-synaptic membrane of the nerve cell junction (Diehr *et al.*, 1991). It is systemic and thus can provide a broader spectrum of control than tefluthrin. As with tefluthrin, it is applied to the outside of pelleted sugar beet seed but at a much higher rate (90 g AI/unit). It gives good control of a wide range of soil and foliar pests in sugar beet and other crops (Eibert *et al.*, 1991). Against soil pests in the UK in 1990 and 1991 imidacloprid gave poorer control of 'the soil pest complex' than tefluthrin (Table 3), but, where pygmy beetles (*Atomaria linearis*) have been a major problem, imidacloprid has performed better, particularly on the continent (Altmann, 1991; Heatherington & Bolton, 1992; Dewar, 1992a). This was probably due to the additional systemic protection afforded by imidacloprid to the cotyledons and young true-leaves of seedlings which were attacked by immigrating adult beetles (Dewar, 1991; A. Wauters in preparation).

This systemic property has resulted in good control of other foliar pests, such as the leaf-mining larvae of mangold flies (*Pegomya hyoscyami*), flea beetles (*Chaetocnema tibialis*) and the aphids, *Myzus persicae* and *Aphis fabae* (Altmann, 1991; Heatherington & Meredith, 1992; Heatherington & Bolton, 1992; Dewar *et al.*, 1993; Schmeer *et al.*, 1990). Its effects on aphids are particularly noteworthy; imidacloprid has prevented or reduced aphid colonisation of beet for up to 10 weeks after sowing, giving as good or better protection than the best of the aphicidal granules, aldicarb (Dewar & Read, 1990; Dewar, 1992; Dewar *et al.*, 1993) (Fig. 1). The consequences of this latter activity have been reduced infection with virus yellows (Table 4). In laboratory experiments, transmission of the semi-persistent beet yellows virus

TABLE 3. Effects of insecticidal seed treatments (ST) or granules (Gr) on sugar-beet seedling establishment, 1990-91.

Treatment	Rate (AI/unit or AI/ha)	Seedling establishment (%) at				Mean (4 trials)
		Ramsey 1990	Baston Fen* 1990	Ramsey 1991	Thorpe Tilney 1991	
Untreated	-	30	19	80	88	54
Tefluthrin ST	10	69	35	96	88	72
Imidacloprid ST	90	51	27	86	88	63
Aldicarb Gr	760	53	29	87	90	65
Carbofuran Gr	600	54	41	81	88	66
LSD (5%)		19.3	12.4	10.3	4.9	

*poor soil conditions at drilling

(BYV) by caged infective *M.persicae* was not prevented, but transmission of the persistent luteovirus, beet mild yellowing virus (BMYV) was substantially reduced suggesting that normal feeding behaviour was interrupted by the insecticide (Dewar *et al.*, 1992).

Imidacloprid gives the same environmental benefits as tefluthrin - lower rates of AI applied to soil, specific placement of AI around seed, and few adverse effects on non-target organisms (Pflüger & Schmuck, 1991). It is however more expensive, as would be expected for a chemical with wider activity, which may affect its sales in the market place.

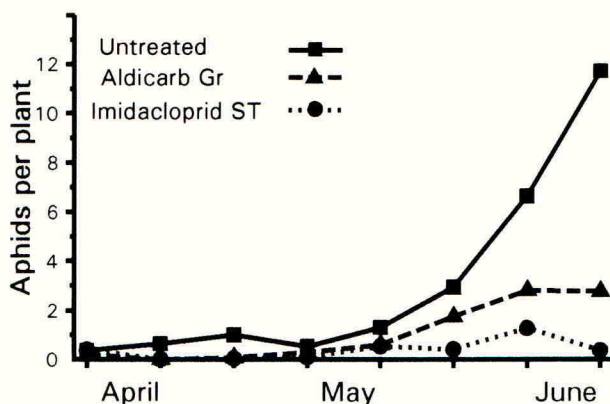


FIGURE 1. The effect of imidacloprid seed treatment and aldicarb granules on aphids in sugar beet : Sandy, Bedfordshire 1993.

TABLE 4. The effect of imidacloprid seed treatment (ST) and aldicarb granules (Gr) on the incidence of virus yellows in sugar beet in four trials in 1990 and 1993.

Treatment	% plants infected (arcsin) at				Mean (4 sites)
	Sandy 12.7.90	Clacton 26.7.90	Sandy 24.8.93	Terrington 17.9.93	
Untreated	56.0(69)*	24.9 (18)	24.8(18)	18.8(10)	31.1(27)
Aldicarb Gr	42.7(46)	16.7(8)	22.8(15)	12.3 (5)	23.6(16)
Imidacloprid ST	29.2(24)	14.3(6)	14.7(6)	7.3(2)	16.4(8)
LSD(5%)	3.9	3.9	4.1	10.9	

*Figures in brackets are backtransformed to % plants infected.

Imidacloprid received approval for use in the UK in late 1993, and will be used extensively for the first time in the 1994 crop. It is already available in France, Belgium, the Netherlands and Finland where 50%, 62%, 12% and 2% respectively of the national crop was treated in 1993. Indeed, the uptake of the product by growers in France and Belgium has been so rapid that sales of other insecticides, such as granules and aphicide sprays, have fallen dramatically. In the UK, the use of tefluthrin in 1991 on 21% of the beet area resulted in an 18% reduction in area treated with carbamate granules and lindane sprays (Dewar & Asher, 1993). It remains to be seen what impact imidacloprid will have in 1994, but it must be a major benefit for the environment that the introduction of these new seed treatments is achieving the objective of substantial reduction in pesticide use.

INTERACTIONS BETWEEN CHEMICALS

Previous work on the incorporation of carbamate insecticides in the pellet along with the fungicide hymexazol demonstrated adverse interactions between the two chemicals, resulting in a reduction in their extractability and efficacy (Asher & Payne, 1989; Heijbroek, 1989). No adverse interactions were observed between hymexazol and either tefluthrin or imidacloprid when seeds were sown in soil infested with *A. cochlioides*, and maintained at 22°C (Table 5). Hymexazol gave very good control of the disease wherever it was applied.

Table 5. Effect of insecticide seed treatments on the control of *A. cochlioides* by hymexazol in the seed pellet.

Insecticide	Rate AI/unit	Hymexazol	Percent plants infected
—	—	—	67.4
—	—	+	2.2
Tefluthrin	10	—	62.6
Tefluthrin	10	+	6.2
Imidacloprid	90	—	50.2
Imidacloprid	90	+	2.5
LSD (5%)			9.9

CONCLUSIONS

The use of fungicidal seed treatments is now the only way of controlling seedling diseases in most countries in Europe, and use of insecticidal seed treatments is becoming more popular as better, more active chemicals become available. This trend is accompanied by a large reduction in use of granule and spray formulations, which are often applied at much higher rates. The development of seed treatments in sugar beet has thus contributed to a major reduction in pesticide use with consequent benefits to the environment.

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REFERENCES

- Altmann, R. (1991) Gaucho - a new insecticide for controlling beet pests. *Pflanzenschutz - Nachrichten Bayer* **44**(2), 159-174.
- Asher, M.J.C.; Payne, P.A. (1989) The control of seed and soil-borne fungi by fungicides in pelleted seed. *Proceedings of 52nd Winter Congress of the Institut International de Recherches Betteravieres*, 179-193.
- Bruin, G.C.A.; Edgington, L.V. (1983) The chemical control of diseases caused by zoosporic fungi. In *Zoosporic Plant Pathogens* (Ed. S.T. Buczacki), Academic Press, London, 352 pp.
- Cooke, D.A.; Winder, G.H.; Prince, J.W.F.; Ecclestone, P. (1991) Tefluthrin: a new seed treatment to protect sugar beet crops. *British Sugar Beet Review* **59**(2), 30-32.
- Coulson, J.M.; Brown, R.A.; Edwards, P.J.; Lewis, F.J. (1990) The effects of tefluthrin on terrestrial non-target organisms. *Brighton Crop Protection Conference - Pests and Diseases 1990*, **3**, 975-980.
- Dewar, A.M. (1989) Results of the co-operative trials on pesticides in pelleted seed, 1987-88. *Proceedings of the 52nd Winter Congress of the Institut International de Recherches Betteravieres*, 163-178.
- Dewar, A.M. (1992a) The effect of pellet type, and insecticides applied to pellets on plant establishment and pest incidence in sugar beet in Europe. *Proceedings of the 54th Winter Congress of the Institut International de Recherches Betteravieres*, 89-112.
- Dewar, A.M. (1992b) The effect of imidacloprid on aphids and virus yellows in sugar beet. *Pflanzenschutz-Nachrichten Bayer* **45**(2), 423-442.
- Dewar, A.M.; Asher, M.J.C. (1993) Pest and disease review of 1992. *British Sugar Beet Review* **61**(1), 12-15.
- Dewar, A.M.; Read, L.A. (1990) Evaluation of an insecticidal seed treatment, imidacloprid, for controlling aphids on sugar beet. *Brighton Crop Protection Conference - Pests and Diseases 1990*, **2**, 721-726.
- Dewar, A.M.; Asher, M.J.C.; Winder, G.H.; Payne, P.A.; Prince, J.W. (1988) Recent developments in sugar-beet seed treatments. In: *Applications to Seeds and Soil*, T.J. Martin (Ed), BCPC Monograph No. 39, Thornton Heath: BCPC Publications, pp 265-270.
- Dewar, A.M.; Thornhill, W.A.; Read, L.A. (1990) The effects of tefluthrin on beneficial insects in sugar beet. *Brighton Crop Protection Conference - Pests and Diseases 1990*, **3**, 987-992.

- Dewar, A.M.; Read, L.A.; Hallsworth, P.B.; Smith, H.G. (1992) Effect of imidacloprid on transmission of viruses by aphids in sugar beet. *Brighton Crop Protection Conference - Pests and Diseases 1992*, **2**, 563-568.
- Dewar, A.M.; Read, L.A.; Prince, J.W.F.; Ecclestone, P. (1993) Profile on imidacloprid - another new seed treatment for sugar beet pest control. *British Sugar Beet Review*, **61**(3), 5-8.
- Diehr, H.J.; Gallenkamp, B.; Jelich, K.; Lantzsch, R.; Shiokawa, K. (1991) Synthesis and chemical-physical properties of the insecticide imidacloprid (NTN 33893). *Pflanzenschutz-Nachrichten Bayer*, **44**(2), 107-112.
- Durrant, M.J.; Payne, P.A.; Prince, J.W.F.; Fletcher, R. (1988) Thiram steep seed treatment to control *Phoma betae* and improve the establishment of the sugar-beet plant stand. *Crop Protection*, **7**, 319-326.
- Elbert, A.; Becker, B.; Hartwig, J.; Erdelen, C. (1991) Imidacloprid - a new systemic insecticide. *Pflanzenschutz-Nachrichten Bayer*, **44**(2), 113-136.
- Heatherington, P.J.; Bolton, B.J.G. (1992) Pest control and crop establishment in sugar beet using an imidacloprid-based seed treatment. *Aspects of Applied Biology* **32**, Production and Protection of Sugar Beet, 65-72.
- Heatherington, P.J.; Meredith, R.H. (1992) United Kingdom field trials with Gaucho for pest and virus control in sugar beet, 1989-91. *Pflanzenschutz-Nachrichten Bayer*, **45**(3), 491-526.
- Heijbroek, W. (1989) Interactions between pelleting material, insecticides and fungicides. *Proceedings of 52nd Winter Congress of the Institut International de Recherches Betteravieres*, 213-220.
- Jutsum, A.R.; Gordon, R.F.S.; Ruscoe, C.N.E. (1986) Tefluthrin - a novel pyrethroid soil insecticide. *Brighton Crop Protection Conference - Pests and Diseases 1986*, **1**, 97-106.
- Marrs, G.J.; Gordon, R.F.S. (1988) Seed treatment with tefluthrin - a novel pyrethroid soil insecticide. In: *Applications to Seeds and Soil*, T.J. Martin (Ed.), BCPC Monograph No. 39, Thornton Heath: BCPC Publications, pp 17-23.
- McDonald, E.; Punja, N.; Jutsum, A.R. (1986) The rational design of tefluthrin - a pyrethroid for use in the soil. *Brighton Crop Protection Conference - Pests and Diseases 1986*, **1**, 199-206.
- Moran, A.; Painporay, G.; Cohadon, P. (1988) Improved crop establishment in sugar beet resulting from the use of tefluthrin. *Brighton Crop Protection Conference - Pests and Diseases 1988*, **3**, 997-1002.
- Muchembled, C. (1991) Development of insecticidal treatments in beet. *Pflanzenschutz-Nachrichten Bayer*, **44**(2), 175-182.
- Payne, P.A.; Williams, G.E. (1990) Hymexazol treatment of sugar-beet seed to control seedling disease caused by *Pythium* spp. and *Aphanomyces cochlioides*. *Crop Protection*, **9**, 371-377.
- Payne, P.A.; Asher, M.J.C.; Kershaw, C.D. (1994) The incidence of *Pythium* spp and *Aphanomyces cochlioides* associated with the sugar-beet growing soils of Britain. *Plant Pathology* **43**, in press.
- Schmeer, H.E.; Bluett, D.J.; Meredith, R.H.; Heatherington, P.J. (1990) Field evaluation of imidacloprid as an insecticidal seed treatment in sugar beet and cereals with particular reference to virus vector control. *Brighton Crop Protection Conference - Pests and Diseases 1990*, **1**, 29-36.
- Winder, G.H. (1990) The development of tefluthrin as a new seed treatment for the control of soil-inhabiting pests of sugar-beet seedlings. *Brighton Crop Protection Conference - Pests and Diseases 1990*, **2**, 727-732.
- Winder, G.H.; Dewar, A.M.; Dunning, R.A. (1993) Comparisons of granular pesticides for the control of soil-inhabiting arthropod pests of sugar beet. *Crop Protection*, **12**, 148-154.

REVIEW OF CURRENT AND FUTURE SEED TREATMENT USAGE IN OILSEED RAPE

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ABSTRACT

The seed treatments currently 'Approved' (Anon. 1991) for use on oilseed rape (*Brassica napus*) in the United Kingdom (UK) contain 7 separate active ingredients that were first patented between 17 and 52 years ago. However, in spite of their age, selection of the appropriate product will give adequate control of a) the main seed-borne diseases *Alternaria brassicae* and *Leptosphaeria maculans*, b) the main soil-borne disease, 'damping-off' (*Pythium spp.*), and c) the main seedling pests, flea beetles (*Phyllotreta spp.*), enabling the successful establishment of both winter and spring crops. It is considered desirable that, in the future, new active ingredients need to be developed to supplement or complement both thiram, for the control of 'damping-off', and gamma-HCH for the control of flea beetles.

INTRODUCTION

The 1993 oilseed rape crop is currently estimated at around 410,000 hectares (LMCS, 1993) making it the third largest arable crop in the UK. It is surprising, therefore, that combined fungicide and insecticide seed treatment products for oilseed rape have not previously been reviewed, although fungicide and insecticide seed treatments on crucifers have been discussed (Maude, 1986). The time would appear right, therefore, to carry out a review of available seed treatments.

DISEASE AND INSECT PEST CONTROL WITH SEED TREATMENTS

Target Seed-Borne Pathogens

Seed-borne pathogens of oilseed rape can be a major problem in the UK. In common with crops such as wheat, peas and linseed a cool, wet period between flowering and harvest can result in a high level of pathogens on the seed. If this infected seed is not treated and a cool, wet period follows sowing, particularly if this is late, then severe plant losses can result.

The commonest pathogen in a wet season is dark leaf spot, *Alternaria brassicae* and *A. brassicicola*, with warmer conditions favouring the development of the latter. Warm, moist conditions in May-July give rapid development, with the spores being dispersed by wind and rain splash up the plant onto the pods and

which then infect the seed.

Sowing untreated, infected seed can result in reduced emergence due to plant death, similar to 'damping-off', and occasionally to wirestems (thin black stems). Infections on cotyledons and young leaves give a source of infection for subsequent leaves or adjacent plants. Serious infections of *Alternaria* have not occurred since the early 1980's, although some infection was found in 1992 on swathed crops (J.E. Thomas, personal communication). There do not appear to be any resistant varieties in commercial use in the UK.

Leaf spot and stem canker, caused by *Leptosphaeria maculans* is a very common disease of oilseed rape. The source of inoculum may be infected seed bearing pycnidia, or stubble bearing pseudothecia which release ascospores in the autumn to early spring. Two strains of *L. maculans* are recognised. Both may cause leaf spots, commonly referred to as Phoma leaf spot, but, typically, only the aggressive strain grows systemically into the leaf petiole and eventually produces a cankered stem. Several oilseed rape varieties in commercial use have moderately high levels of resistance to stem canker (Anon. 1993). Infected seed can occasionally kill seedling plants and spread the leaf spot phase to oilseed rape growing in primarily uninfected areas.

Target Soil-Borne Pathogens

Clubroot (*Plasmodiophora brassicae*) is potentially a serious soil-borne disease for all brassicae. It occurs in many oilseed rape growing areas, but predominantly in the west and north. There are no resistant varieties of oilseed rape, but some spring forage rapes have partial resistance.

'Damping-off' (*Pythium spp.* and *Rhizoctonia solani*) can occur in the UK but may also be due to *Alternaria*, *Phoma* and other soil-borne diseases. *Pythium* may occasionally cause wilting and death of young plants. *Rhizoctonia* can also cause problems at the seedling stage, but it is associated with premature ripening of more mature plants in Canada, and has been reported as causing similar symptoms in France and Germany. Its importance in the UK is not known. In Canada the *R. solani*, anastomosis groups AG2-1 and AG4, have been identified as causing pre- and post-emergence seedling damping off, seedling root rot and basal stem or foot rot (brown girdling root rot) of adult plants (Kataria and Verma, 1992). Generally, the isolates of AG2-1 are more virulent than isolates of AG4, and seedling infection by AG2-1 is favoured by cool weather, whereas warm weather is conducive to severe damping-off by AG4.

Downy Mildew (*Peronospora parasitica*) is a frequently-occurring disease of oilseed rape. The persistent resting stage in the soil infects young plants systemically and severe attacks can occasionally reduce plant population. Conidia produced from primary infections are wind dispersed and lead to further cycles of infection in cool, wet autumns. Many varieties have a high level of resistance to the disease.

Sclerotinia stem rot (*Sclerotinia sclerotiorum*) has become a widespread disease in recent years, with a wide host range, including peas, beans, linseed and potato in addition to oilseed rape. The black sclerotia develop inside the stem and can either drop into the soil, during and after harvest, or contaminate the seed. Developing seedlings can become infected by mycelium when the bottom leaf touches the ground and starts to senesce, this phase of the disease is only important when a wet autumn is associated with high seed or soil infection.

Sclerotinia is the most serious disease in France and Germany and may be a limiting factor to the increase in the growing area of oilseed rape. Some oilseed rape varieties appear to be very prone to infection. However, this appears to be almost entirely due to agronomic factors (Sweet et al, 1992).

Target insect pests

Flea beetles (*Phyllotreta* spp., are the main species) often cause serious damage to newly-emerged seedlings of oilseed rape and other brassicas (Gratwick, 1992). The damage is usually most serious on spring-sown crops, but can occasionally affect crops sown in August. The adult beetles eat holes in the cotyledons and stems of the seedlings, beginning while plants are still below ground, and may check or kill plants, particularly in the spring, when dry weather after emergence causes wilting. Attacks can continue to the first true-leaf stage and beyond, but become progressively less damaging. It has been reported in Canada that early damage caused by *P. cruciferae* and *P. striolata* on oilseed rape delayed plant development, caused unevenness in height and maturity, reduced seed yield and raised the chlorophyll content of the seed (Lamb, 1984).

Cabbage stem flea beetle (*Psylliodes chrysocephala*) attacks only winter oilseed rape. The adult beetles move into crops in August and September and feed on leaves, causing holes and occasionally killing young plants, particularly in dry weather. Eggs are laid in the soil over several weeks and the larvae enter the plants from October to April, tunnelling in stems and leaf petioles. This flea beetle has become widely distributed.

Rape winter stem weevil (*Ceutorhynchus picitarsus*) has a life history very similar to that of cabbage stem flea beetle. Plant attacks in September/October cause only insignificant damage, but plants severely attacked by larvae during the winter can be killed and less damaged plants stunted with a rosette like appearance.

Cabbage root fly (*Delia radicum*) is a serious pest of brassica crops in the UK. However, its effect on oilseed rape is largely ameliorated by the imperfect fit of life cycle and cropping systems. Eggs are laid in three generations - the first during mid- to late-April, the second and third generations usually overlap, so that egg laying can occur during July, August and September. The first generation can coincide with late-drilled spring, and the third generation with early-drilled

winter oilseed rape. The eggs hatch into maggots, large numbers of which, feeding on roots, can virtually kill plants.

Seed-borne disease control

All oilseed rape seed sold by seed companies in the UK must by law be certified that it is free from disease, i.e. *Phoma* (*Alternaria* is not, at present, part of the Certification Scheme) (Anon., 1993a). Advisory tests, carried out by NIAB on brassicae seeds in 1992/93, showed that, of 39 samples tested for *Alternaria*, 73.3% were infected, and, of 36 samples tested for *Phoma*, 24.3% were infected (Reeves and Simpkins, 1993). Virtually all oilseed rape seed is treated with a fungicide/insecticide seed treatment - 94.6% was treated in 1990 (Davis et al, 1990).

Table 1 gives the seed treatments registered in the UK and Table 2 the seed treatments registered and hectares grown in some of the main producing countries.

TABLE 1. Oilseed Rape - fungicide and insecticide seed treatments registered in the U.K.

Active Ingredients (Tradename)	AI g/l or g/kg	Formu- lation	Rate/100kg Product	seed g AI	Target Diseases and Pests
Metalaxyl (Apron 350 FS)	350	FS	285ml	100	Damping-off Downy Mildew
Gamma-HCH Thiram (Hydraguard)	533 200	FS	1500ml	800 300	Flea beetle Damping-off
Gamma-HCH Thiram Thiabendazole (Hysede FL)	400 140 120	FS	2000ml	800 280 240	Flea beetle, Damping-off <i>Phoma</i>
Gamma-HCH Thiram Fenpropimorph (Lindex Plus FS)	545 73 43	FS	2200ml	1200 161 95	Flea beetle Damping-off <i>Phoma</i> <i>Alternaria</i>
Iprodione (Rovral WP)	500	WP	500g	250	<i>Alternaria</i>
Gamma-HCH Thiram Carboxin (Vitavax RS)	675 90 45	FS	2200ml	1485 99	Flea beetle Damping-off <i>Phoma</i>

TABLE 2. Seed treatment active ingredients registered on oilseed rape in some of the main producing countries.

Country	ha (x1000) grown in 1992	Active ingredients registered
Canada	4,178	gamma-HCH/thiram/carboxin gamma-HCH/iprodione gamma-HCH/thiabendazole/thiram
Denmark	191	gamma-HCH/thiram/carboxin
France	686	isofenfos/thiram, metalaxyl, methiocarb, thiram
Germany	975	carbosulfan, isofenfos/thiram
Poland	350	carbendazim/thiram, carboxin/thiram isofenfos/thiram, thiram

The control of *Phoma* with carboxin has been reported (Kharbanda, 1989); that of *Phoma* and *Alternaria* with thiabendazole and thiram (Maude et al, 1984) and with fenpropimorph, iprodione and thiram (Maude and Suett, 1986). Further evidence that gamma-HCH/thiram/carboxin will give a commercially acceptable level of control of *A. brassicae* comes from a Uniroyal Chemical Ltd. contract trial carried out by NIAB (Table 3). Here seed was used that had been artificially inoculated during flowering in 1992. Individual seeds were sown, in compost, in 154 modules x 6 replicates and grown in the glasshouse. Only 3 replicates were assessed for *A. brassicae*.

TABLE 3. Oilseed rape emergence and % control of *Alternaria brassicae* on seedlings at one true leaf.

Treatment	Rate/100 kg seed g AI ml FP	% Control of	
		% Emergence 19.10.92	% Control of <i>Alternaria</i> 04.11.92
Untreated (% infection)		94.9 a	(2.6) a
Gamma-HCH/thiram/ carboxin	743/99/50 1100	90.9 a	100.0 b
Gamma-HCH/thiram/ carboxin	1485/198/99 2200	93.9 a	78.3 b
Gamma-HCH/thiram/ fenpropimorph	1200/161/95 2200	93.7 a	88.9 b
LSD (P=0.05)		3.765	51.7

Soil-borne disease control

The control of the 'damping-off' complex (*Pythium spp*) is achieved with products containing thiram or metalaxyl. In soil inoculated with *Pythium*, seed treatment with either thiram or metalaxyl + captan gave control of pre-emergence 'damping-off' of Brussels sprout and cabbage seedlings. No post-emergence 'damping-off' occurred in these crops or in oilseed rape following treatment with metalaxyl + captan whilst post-emergence losses from untreated seed ranged from 10.2-19.4% and from thiram-treated seed from 5.7-7.4% (White et al., 1984). The effect of adding metalaxyl to gamma-HCH/fenpropimorph (reduced rate of fenpropimorph) compared with standard rate gamma-HCH/thiram/fenpropimorph, resulted in further increases in the spring stand count and yield, and gave additional control of downy mildew (Smith and Margot, 1987). Metalaxyl-treated crops appeared to be more resistant to winter kill than those treated with the standard. This was believed to be related to reduced autumn downy mildew infection and/or *Pythium* control allowing improved rooting of plants going into the winter.

In Canada the control of pre-emergence damping-off and post-emergence seedling root rot caused by *Rhizoctonia solani* AG-2-1 and AG-4 with carboxin, iprodione, thiabendazole and other products has been demonstrated in growth chamber tests (Kataria and Verma, 1993).

There are no label recommendations for the control of *Sclerotinia*.

Insect pest control

Only one insecticide is registered in the UK for the control of flea beetles, namely gamma-HCH. Rates of use range from 800, through 1,200 to 1,485 g AI/100 kg. Contract field trials for Uniroyal Chemical Ltd., carried out by ADAS in Herefordshire and Lincolnshire on spring oilseed rape, have shown that gamma-HCH/thiram/carboxin and gamma-HCH/thiram/fenpropimorph gave significant reductions in plant damage from *Phyllotreta undulata* (Green et al., 1993). Confirmation is provided by data from a further contract trial, by ADAS at Rosemaunde, Herefordshire, in spring 1993 (Table 4). Spring oilseed rape, cv. Tanto, treated at standard rate by Uniroyal Chemical Ltd., was drilled in plots 12 x 2.2m x 3 replicates on 15.4.93. Assessments were made on 18.5.93 at growth stage 1-02.

In Canada a yield increase of 61.9% was recorded on oilseed rape treated with gamma-HCH at 1,560 g AI/100kg, compared with a yield increase of 6.7% following a spray treatment of azinphos - methyl for the control of flea beetles (Westdal et al, 1975).

No insecticide seed treatments are currently registered for use against cabbage stem flea beetle. However, furathiocarb 25 and 50 g AI/kg seed, applied as a film coating seed treatment to oilseed rape, has given significant reduction in the number of larvae per stem at two sites (Salter and Smith, 1987).

TABLE 4. Oilseed rape emergence and damage to cotyledons from *Phyllotreta undulata*.

	Mean No. plants/m ²	Mean % plants damaged*	Mean % damage to cotyledons*
Untreated	135.6 a	74.3 b	21.0 c
Gamma-HCH/thiram/carboxin	152.3 a	45.8 a	7.8 ab
Gamma-HCH/thiram/fenpropimorph	153.1 a	51.9 a	4.9 ab
SED (12DF)	(8.63)	(8.30)	(2.85)

*data transformed using angles

Non-chemical control

Very little biological control work has been carried out. However, it has been observed that indigenous populations of *Pseudomonas fluorescens*, *Trichoderma harzianum* and the non-pathogenic binucleate *Rhizoctonia*-like forms have demonstrated a certain level of control against the virulent isolates of *R. solani* (Kataria and Verma, 1992).

Cultivar selection can be used to reduce crop susceptibility to the foliar disease *Leptosphaeria*, which must, in turn, reduce seed-borne infections. There is no published information on cultivar resistance to 'damping-off' or to flea beetle attack.

Status of current products and possible future introductions

Fungicides

Thiram, first reported in 1942 (Worthing and Hance, 1991), was not expected to survive into the 1990's. It has had its toxicology defended by a taskforce, including Uniroyal Chemical Inc., and, following a review by the FAO/WHO Joint Meeting on Pesticide Residues in 1992, the committee has re-established an Acceptable Daily Intake for thiram assuring its seed treatment uses for the present. The only alternative to thiram for the control of damping-off is, at present, metalaxyl which, while it has the advantage of giving additional control of downy mildew, does have the disadvantage of being more expensive than thiram. It is not known of any compounds being developed for this use, but it is hoped that some will be. Fenpropimorph does not appear to have any registration issues.

Several new seed treatment fungicides have been registered on cereals in recent years, but the only one which is likely to be registered on oilseed rape, for the control of *Alternaria* and *Leptosphaeria*, is fenpiclonil (Nevill et al, 1988) or another phenylpyrrole.

A new sequential co-application of gamma-HCH/thiram and iprodione is likely to be marketed when 'Approval' is granted.

The development of new products that would control club root and *Sclerotinia* as well as damping-off and downy mildew would be an added bonus.

Insecticides

Gamma-HCH, which was first reported in 1945 (Worthing and Hance, 1991), is no longer used in some countries such as France, Germany and Sweden. At present its future in the UK is not at risk, but it would be desirable, both from the registration point of view and to obtain a higher level of control of flea beetle, to find an alternative active ingredient. Iodofenphos is registered for this use in France and Germany, but not in the UK. The very active imidacloprid is unlikely to be developed on oilseed rape because of insufficient activity against flea beetle. However, synthetic pyrethroids are showing promise as seed treatments (Uniroyal Chemical Ltd., unpublished data).

The ideal seed treatment insecticide would be systemic to give more persistent control of *Phyllotreta* spp. and also to give control of *Psylliodes* and *Ceutorrhynchus*.

SEED COATINGS

Of all the major crops grown in the UK, oilseed rape is the one most commonly coated with coloured polymer coatings during the seed treatment process. The advantages of seed coating has been well summarised (Halmer, 1988). Several factors have accounted for their high uptake on oilseed rape including - the low seed rate/ha which reduces cost and the fact that the main product used (gamma-HCH/thiram/fenpropimorph) and also gamma-HCH/thiram/thiabendazole contain no dyes or pigments, so that the addition of a coloured coating gives a more attractive seed sample.

The future for oilseed rape seed treatments

The forecast areas of oilseed rape for 1993, 1995 and 1997 (LMCS, 1993) are given in Table 5.

TABLE 5. Hectarages of oilseed rape grown in 1991, with 1992 and forecasts for 1993-1997 expressed as a percentage of 1991 figures.

	ha (x1000)		ha expressed as % of 1991		
	actual 1991	1992	1993	1995	1997
Denmark	276	69.2	63.0	61.6	58.0
France	735	93.3	73.7	65.2	51.6
Germany	944	103.3	64.3	46.1	38.9
UK	441	95.7	93.2	73.9	71.7
EEC total	2,444	94.6	85.1	67.8	59.4

By 1997 the EEC total hectareage of oilseed rape is forecast to fall by 40% compared with a forecast fall of 28% for the UK which is cushioned by their ability to switch to growing the cheaper spring crop. Whilst the quantity of seed treatments sold will obviously decline, their usage on virtually all the crop will no doubt continue due to their high cost-effectiveness.

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REFERENCES

- Anonymous (1991) 1991 Product Guide to Seed Treatments available in the UK, Ed. D. Soper, BCPC, 11.
- Anonymous (1993) Varieties of Oilseed Crops 1993. Farmers Leaflet No. 9. NIAB, 6.
- Anonymous (1993a) Oil and Fibre Plant Seed Regulations 1993. HMSO, SI 2007, 18.
- Davis, M.P.; Garthwaite, D.G.; Thomas, M.R. (1990) Pesticide usage survey report 85. MAFF, 10.
- Gratwick, M. (1992) Crop pests in the UK. Chapter 34 Flea beetles. Chapman and Hall, London, 173-175.
- Green, D.B.; Holliday, M., Bartlett, D.H. (1993) *Tests of Agrochemicals and Cultivars*, No. 14 (*Annals Applied Biology* 122 supplement), 18-19.
- Halmer, P. (1988) Technical and commercial aspects of seed pelleting and film-coating. In: *Applications to Seeds and Soil*, T.J. Martin (Ed.) BCPC Monograph No. 39, Thornton Heath: BCPC Publications, 191-204.
- Kataria, H.R.; Verma, P.R. (1992) *Rhizoctonia solani* damping-off and root rot in oilseed rape and canola. *Crop Protection*, 11 (1), 8-13.
- Kataria, H.R.; Verma, P.R. (1993) Efficacy of fungicidal seed treatments against pre-emergence damping-off and post-emergence seedling rot of growth chamber grown canola caused by *Rhizoctonia solani* AG-2-1 and AG-4. *Canadian Journal of Plant Pathology*, 12, 409-416.
- Kharbanda, P.D. (1989) Effectiveness of fungicides to control blackleg of canola rapeseed. *Canadian Journal Plant Pathology*, 11 (2), 193.
- Lamb, R.J. (1984) Effects of flea beetles, *Phyllotreta* spp., on the survival, growth, seed yield and quality of canola, rape and yellow mustard. *Canadian Entomologist*, 116 (2), 269-280.
- LMCS Landel Mills Commodity Studies (1993). *European Agrochemical Monitor*, Proprietary Study.
- Maude, R.B.; Humpherson-Jones, F.M.; Shoring C.G. (1984) Treatments to control *Phoma* and *Alternaria* infections of brassica seeds. *Plant Pathology*, 33, 525-535.
- Maude, R.B. (1986) Treatment of vegetable seeds. In: *Seed Treatment*, K.A. Jeffs (Ed) Thornton Heath: BCPC, 239-261.

- Maude, R.B.; Suett, D.L. (1986) A review of the progress in the development of seed treatments for the control of *Alternaria* and *Phoma* infections of brassica seeds. *Aspects of Applied Biology*, **12**, *Crop Protection in Vegetables*, 13-19.
- Nevill, D.; Nyfeler, R.; Sozzi, D. (1988) CGA 142705: A novel fungicide for seed treatment. *Brighton Crop Protection Conference - Pests and Diseases 1988*, **2**, 65-72.
- Reeves, J.C., Simpkins, S.A. (1993) The incidence of some seed-borne diseases in the UK. In: *Plant health and the European single market*, D. Ebbels (Ed.), *BCPC Monograph No. 54*, Thornton Heath: BCPC Publications, 333-338.
- Salter, W.J.; Smith, J.M. (1987) Furathiocarb seed coatings: potential replacements for topical pesticide applications. In: *Applications to Seeds and Soil*, T.J. Martin (Ed.), *BCPC Monograph No. 39*, Thornton Heath: BCPC Publications, 277-283.
- Smith, J.M.; Margot, P. (1987) The use of metalaxyl as a seed or soil treatment. In: *Applications to Seeds and Soil*, T.J. Martin (Ed.), *BCPC Monograph No. 39*, Thornton Heath: BCPC Publications, 41-53.
- Sweet, J.B.; Pope, S.J.; Thomas, J.E. (1992) Resistance to *Sclerotinia sclerotiorum* in linseed, oilseed rape and sunflower cultivars, and its role in integrated control. *Brighton Crop Protection Conference - Pests and Diseases 1992*, **1**, 117-126.
- Westdal, P.H.; Romanow, W.; Askew, W.L. (1975) Annual Conference Manitoba Agronomists December 16 and 17, 1975, 55-57.
- White, J.G.; Crute, I.R.; Wynn, E.C. (1984) A seed treatment for the control of *Pythium* damping-off diseases and *Peronospora parasitica* on brassicas. *Annals of Applied Biology*, **104**, 241-247.
- Worthing, C.R.; Hance, R.J. (1991) *The Pesticide Manual*, **9**, 452 and 822.

DIAGNOSTIC METHODS FOR THE DETECTION OF PLANT PATHOGENS IN VEGETABLE SEEDS

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ABSTRACT

Diagnostic methods to detect plant pathogens associated with vegetable seeds are applied for various reasons at different stages in the seed production chain. The reason for testing, however, may differ from that for which a particular method had been originally developed. The consequence of applying an unsuitable method is that incomplete or even false information is obtained on the health status of the seed.

In this paper an overview is presented on the various objectives of seed health testing, the principles of the available methods and the choice of the most appropriate method. Factors which might interfere with the diagnostic value of the test results are considered. Finally, some factors relevant to the correct evaluation and interpretation of the analytical data are reviewed.

INTRODUCTION

A world wide increase in the production area of vegetable seeds and a dramatic expansion of the international seed trade has enhanced the interest in diagnostic methods for detection of pathogenic fungi, bacteria and viruses associated with seeds. The occurrence of such plant pathogens in vegetable seeds has been described over the years by many authors. The most recent updated compilation of this information was presented by Richardson (1990) as section 1.1 of the Handbook on Seed Health Testing of the International Seed Testing Association (ISTA).

Reasons for analysing seeds for the presence of plant pathogens can be diverse and are related to the stage in the production process at which the seed needs to be tested. The purpose of testing, the nature of the pathogen and the level of contamination that can be tolerated will determine the methodology to be followed.

Detection methods have been described in the literature for a wide range of seed-borne pathogens. Detailed descriptions for detection of seed-borne fungi and some bacteria and viruses can be found in the Working Sheets of the ISTA Handbook on Seed Health Testing (1981). Excellent technical information for detection of bacteria in seeds is given by Saettler *et al.* (1989) and, for their identification by Schaad (1988). Serological methods which can be used for detection and identification of viral and bacterial plant pathogens were described by Hampton *et al.* (1990).

The users of seed health testing methods are often not aware of the fact that the analytical data obtained do not always provide the information which is needed in a given situation. This paper aims to evaluate the various situations which may lead to the application of diagnostic methods on seed. The benefits of testing at different stages in the seed production process, the kind of methods that are available and the limitations that

can be expected in the analysis and interpretation of the results are also considered.

PURPOSE OF TESTING

Testing of seeds for 'health' or contamination with plant pathogens can be performed at several stages, as illustrated in Figure 1. The reasons for testing can vary at each stage.

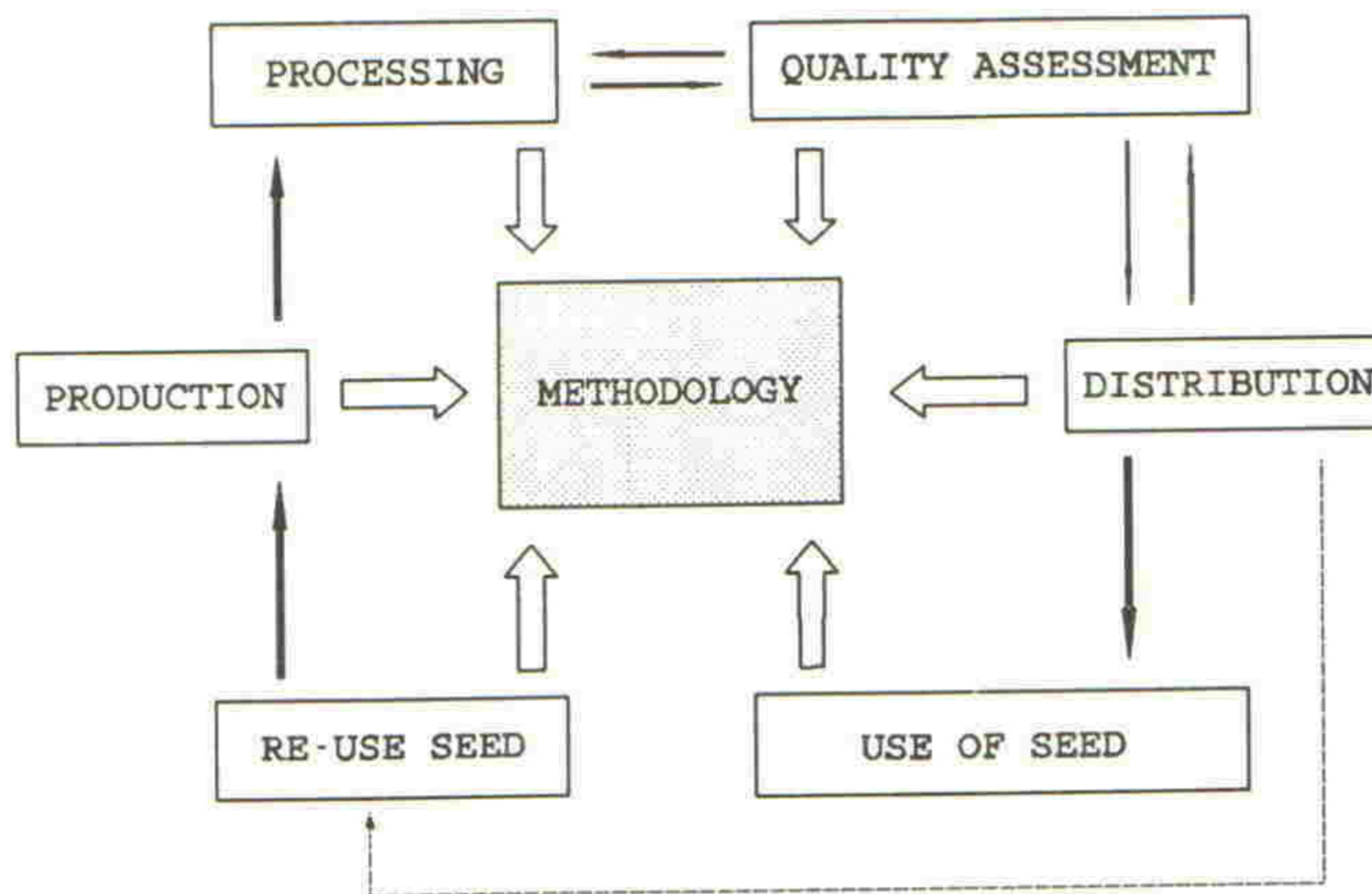


Figure 1. Scheme representing the various stages at which seed health testing may be useful.

Production

Unripe or almost ripe seed can be collected from the seed crop during the period the seed is formed and matures, and analysed for different reasons. Analytical data may confirm transmission of a pathogen to the seeds from diseased plants found in the crop itself or in adjacent fields. Such information might be useful for curative or preventative control measures to be undertaken prior to, or following harvest.

Pre-harvest treatment of the crop

Sprays with chemicals or biological agents may prevent further contamination or infection of seeds with pathogenic fungi. Excessive contamination of seed with certain pathogenic fungi, bacteria and viruses may be prevented by early harvesting. Of course, a balance has to be struck between the required maturity of the seed and the level of contamination with the pathogen that can be tolerated.

Adaption of the post-harvest conditions

Quick drying of threshed or unthreshed seed may prevent further spread of fungi or bacteria through the bulk of material. Development of embryonic or endosperm infections which are more difficult to control may be stopped in time. Storage at low temperatures after drying may have a similar effect.

Planning of the processing

Advanced knowledge of the occurrence of seed-borne pathogens in a seed lot can be used in different ways. One might avoid cross contamination between diseased and healthy seed lots during the various processes of threshing, cleaning, grading or any treatment given afterwards, especially, when one of these processes involves seeds passing through a wet or damp phase of handling. Fungi such as *Phoma* and *Septoria spp.* may release enormous amounts of conidia in water from pycnidia associated with the seed coat. Foreknowledge also allows the planning of the most appropriate curative treatment, which can be chemical, physical or biological.

Planning of the sales

Finally, early knowledge of the presence of a plant pathogen in a seed lot will contribute to better sales management, especially when this pathogen is subject to the phytosanitary regulations of the country of final destination of the seed.

Processing

It is worthwhile drawing representative samples from each seed lot prior to processing, and to analyse them before further steps are undertaken. A quick visual inspection of the dry seed may sometimes indicate the presence of a pathogen. Examples of this are the occurrence of sclerotia of *Sclerotinia sclerotiorum*, presence of dark coloured spots on seeds of *Phaseolus vulgaris* and *Pisum sativum* caused by *Colletotrichum lindemuthianum* and *Ascochyta spp.* respectively. In other cases, extraction or incubation procedures will be necessary for an effective diagnosis. On the basis of such diagnosis, which should preferably be a quick one, the most optimal processing can be planned. Attention can then be paid to the following substages.

Cleaning

Mixing of 'healthy' and 'diseased' lots prior to any cleaning process should be avoided. Additional processes can be needed for removal of sclerotia or seeds with spots or discolorations caused by pathogens. Liquid cleaning may cause smearing of certain pathogens and, under these circumstances, should not be used.

Grading

The prevalence of certain pathogens may be linked to sublots consisting of seeds of a certain size. It is known for example that the lightest seeds of *Cucumis melo* are more often infected with squash mosaic virus than the heavier seeds (Middleton, 1944).

Storage

The viability of several pathogens, for example *Septoria apiicola* in celery seed (Mujica, 1943), decreases with time. Knowledge about such contamination may postpone further steps until the risk of seed transmission after sowing has become acceptably low.

Physical treatment

Treatments of seed lots by heat, mostly given as a hot water treatment, may kill a target pathogen in the seed, but negative effects on the viability of the seed may be one of the other consequences. Reliable pre-screening for the presence of the pathogen will avoid unnecessary loss of quality in those lots which are 'free' of the pathogen.

Physiological treatment

Treatments such as osmo- or hydropriming are often applied on vegetable seeds in order to improve the sowing value. In addition, pelleting the seed may give further improvement. However, such expensive modification of seeds becomes worthwhile only when there is a guarantee that no cross contamination with pathogens takes place during one of the technological processes. Consequently, rather sensitive health tests are needed, which give information about such risks.

Chemical treatment

Most vegetable seeds are treated nowadays with one or more chemicals. The choice of these chemicals should of course be targeted at the pathogens which are associated with a particular seed lot. In practice however, all lots of a particular seed species often get the same standard treatment though the kind of contamination may differ in different lots. In particular the use of selective chemicals such as the benzimidazoles or, for example, iprodione requires caution. Treatment with iprodione of carrot seed contaminated with both *Alternaria* and *Fusarium spp.* gives lower quality seed, as *Fusarium* will not be controlled and may even be stimulated. Treatment of such a seedlot with carbendazim causes an opposite effect. A diagnostic method indicating the level of contamination and specifying the fungal contaminant, at least at genus level, is a prerequisite.

Biological treatment

Interest in the treatment of seeds with biological control agents (BCA's) has increased since the application of certain chemicals may be prohibited in the future, in order to protect the environment. Results however are still poor in practice. As has been mentioned for chemical treatment, knowledge of the composition of the natural mycoflora on the seed may be of relevance as interactions between the added BCA and the mycoflora cannot be excluded and may vary between seed lots.

Quality assessment

Knowledge of the health status of seed lots which have reached the stage of distribution is the most obvious reason for testing. The final destination of the seed lot, the way it is distributed to the user and the phytosanitary or quarantine regulations being in force determine the choice of the method. Based on the test result it is possible that the seed may have to be treated chemically, physically or biologically in order to meet national quality standards or those of the potential user of the seed. In such cases a second test may be required to verify or to measure the efficacy of the treatment. The method used will depend on the nature of the treatment given. When biologically active residues, e.g. fungicides, bactericides or BCA's have been applied to the seed, traditional microbiological techniques will generally not be suitable; neither will serological or other modern molecular techniques based on DNA or RNA analysis. A growing-on test may solve this problem.

Distribution

Seed lots will generally meet the quality standards of the marketing company, and those of the Nation or State in which they are finally processed and made ready for sale. Additional, and often different, requirements for certain pathogens may arise at the moment export takes

place. The method of processing and the treatment that was applied will then determine whether testing for the presence of such pathogens is still possible and reliable. Analytical data from tests carried out at an earlier stage during seed production and processing may be useful to convince the client that the seed lot meets all requirements set by phytosanitary or quarantine regulations.

Use

Health tests are normally not necessary once the seed has reached its destination, viz. the retailer in an importing country or the grower of a vegetable crop, unless a check has to be carried out by law in order to meet the requirements for an Import Permit. Again, difficulties will arise when the pathogen is detectable in such a situation and it cannot be proved that the seed has been treated in such a way that the risk of disease transmission is negligible. It is often not realised, however, that the sensitive methods based on serological and molecular techniques that are now available are not suitable as they do not necessarily indicate survival of the target pathogens. The only reliable test would then be a culture method or a growing-on method, showing specific symptoms of the disease after imbibition of the seed.

Re-use

Decisions have to be made at this stage about the use of seed for further multiplication or for long term storage in genebanks. Consequently, the requirements set for disease 'freedom' should be as stringent as possible. Health tests performed in earlier stages may already have given enough information; otherwise, very sensitive and if possible, non-destructive tests have to be carried out to confirm the absence of the target pathogen.

PRINCIPLES OF DETECTION METHODS

Methods for the detection of seed-borne fungi, bacteria and viruses have been reviewed by Neergaard (1977) and Agarwal & Sinclair (1987). Official testing procedures for detection of fungi can be found in the Rules of the ISTA (International Seed Testing Association, 1993). These methods are recommended for official certification purposes provided that the seeds submitted for testing are not treated with chemicals and have been obtained according to a standardised sampling procedure. Other methods for fungi and for a number of seed-borne bacteria and viruses are presented in a series of separate Working sheets (ISTA, 1981- onwards). They have been evaluated by the Plant Disease Committee of the ISTA for reproducibility. In a number of sheets an indication is given of the diagnostic value of the method(s) described.

Direct inspection of the seed or seed washings

The most simple procedure by which seed infection through fungi, bacteria or viruses can be determined is by inspection of the dry seed with the naked eye or with the help of a microscope. One may inspect for presence of sclerotia, specific discoloration or malformations. Fungi with characteristic conidia or spores can also be detected on dry seed or in seed washings. One should realise, however, that symptomless seed may still carry slight and latent infections.

Growing-on tests

Another generally applicable procedure for a number of fungi, bacteria and viruses is the so-called growing-on test in which seeds are incubated in an artificial substrate, sand or soil in order to induce specific symptom development in the seedlings. This kind of test may also be needed to confirm, for example, the efficacy of an eradicated seed treatment against a bacterium or a virus. The possibility that some seeds may be unable to germinate because of lethal infections should not be overlooked.

The most common tests for fungi, bacteria and viruses

Most commonly applied methods for fungi are based on incubation of the seed on paper substrates (blotter tests) or on nutrient agar. These procedures are not suitable for detection of bacteria or viruses. These pathogens are now detected most commonly by serological and/or a biochemical techniques as part of the test procedures which are described below.

The test procedure for detection of seed-borne bacteria can include the following steps: a) extraction of subsamples of whole or disrupted seeds in water or buffered solution, b) isolation of the target bacterium from the seed extract by plating the extract or dilutions of it on (semi) selective media, c) identification of suspect colonies via biochemical tests or a serological test with a specific antiserum, and d) testing the pathogenicity of positively identified isolates.

Detection of seed-borne viruses involves a serological test on either extracts of ground seeds or on sap obtained from seedlings raised from seeds. In the first type of extracts both active and inactivated virus particles will be detected; in the latter case information is also obtained on the transmission of active virus, provided remnants of the seed coats are separated from the seedlings prior to extraction. For confirmation of the identity of the virus, additional methods have been reviewed by Agarwal & Sinclair (1987). Newer techniques, such as application of the polymerase chain reaction (PCR), are still awaiting widespread application.

CHOICE OF THE DETECTION METHOD

The choice of which diagnostic method is best suited to analysing a seed lot or sample depends primarily on defining the identity of the target pathogen(s), selecting the optimum sampling procedure for detecting of the pathogen(s) and defining tolerance levels. The sub-sampling procedure, with respect to the number of seeds to be analysed individually or pooled in sub-samples, has to be adjusted to the tolerance level set for the target pathogen (Geng *et al.*, 1983). Choice of a method of detection and sampling also depends on whether qualitative and/or quantitative information is required, e.g. presence or 'absolute' (i.e. 'presumed') absence of a certain pathogen in a seed lot. Information has to be available on how analytical data can be evaluated and interpreted, what kind of conclusions can be drawn from them and what actions have to be taken as a result. The choice of a method may also depend on the skill of the analysts running the tests. Apart from the purpose of the test, the available methods and their principles, it is important to know what logistic and technical facilities are needed to carry out the planned tests (Langerak *et al.*, 1988).

FACTORS INTERFERING WITH THE DIAGNOSTIC VALUE OF THE TEST RESULT

The use of any method must be based on relevant research or practical experience, giving information on how effectively the pathogen in question can be detected. Moreover, one should know how well results can be reproduced and interpreted, recognising the factors which might have been influencing the final test result.

History of the seed lot

At any time, independently of the stage at which a seed crop or lot is sampled for seed health testing, information should be available about: the origin (i.e. the location of production) and the climatological conditions during seed formation, maturation, and at harvest. Similar information is needed on the environmental conditions during transport, storage, processing and treatment of the seeds. Furthermore, it is relevant to have information on any chemical, physical, physiological or biological treatment given to the seed from the moment it is formed. All these factors may influence the location of the pathogen in the seed, the level of contamination and the prevalence of seed infection in the lot, and the chance of detecting the target pathogen.

Sampling and storage of the samples

Samples drawn from a seed lot must be representative of that lot. Directions for correct sampling procedures are given in the Rules for seed testing of the ISTA (1993). One of the prerequisites is that the lot is homogeneous. The size of the sample must be sufficient to ensure that the test results give relevant information. The size of the working sample and the number of seeds taken from it for testing must be closely related to the tolerated infection percentage. Statistical handbooks may be consulted in order to find the optimal sample size. It is very important to dry samples with a high moisture content when the period between sampling and testing exceeds several days, otherwise moulds associated with such seeds may become active. As a consequence, detection of the target pathogen may become difficult or even impossible.

Technical factors

Several factors of technical origin can cause variation in test results. The seeds and associated micro-organisms may respond differently under variable incubation conditions. Therefore, equipment needed for seed health testing must be clean and sometimes even sterile in order to avoid contaminating the material to be incubated. Supervision on correctness of settings for temperature, moisture, light, pressure and cleanness must take place according to a well defined schedule. General guidelines for installations and instruments used in microbiological studies are given in ISO 7218 of the International Organization of Standardization (ISO, 1985).

Materials such as water, filterpaper, sand, soil, agar, nutrient media, chemicals, plates, glassware etc. must be of a standard quality and need regular checks for purity and eventual toxicity for both the seeds and the seed-borne organisms. As the quality of tap water may vary over the year and will, in general, differ from laboratory to laboratory, it is recommended that deionised or distilled water is used. Test material of a biological nature such as antisera, test plants or reference seed samples

need special attention as the risk of quality loss is rather high. Regular checks on quality have to be carried out according to a fixed schedule.

Personnel factors

One of the most important factors in performing seed health tests is the human one. The skills of the personnel determine the success and standardisation of tests. This specially holds for methods in which assessment of characteristics of a biological, and thus variable, nature such as symptoms, colonies of bacteria or fungi and fructifications of fungi are required. It is recommended that such tests be carried out at least in duplicate and are assessed by two analysts. Criteria should be established with respect to accuracy and performance of the test.

EVALUATION AND INTERPRETATION OF TEST RESULTS

It is important that in any laboratory where seed health tests are carried out, comparable results are obtained in repeated testing. A detailed protocol that can be followed has been described by Sheppard in "Guidelines for collaborative study procedures to validate characteristics of a method of analysis" (1993, unpublished), which was adapted from the "Guidelines for collaborative study procedures in chemical analysis" (Anonymous, 1989).

Repeatability and reproducibility of test results

The reproducibility of test methods may vary and different methods may become more or less sensitive to test conditions. It is essential therefore to repeat testing on well-stored reference samples in order to evaluate the precision of test results. The cause of variation should be found and eliminated if reproducibility is lower than originally described and established for the method.

Statistical treatment of analytical data

Regardless of the kind of data that are obtained, a statistical treatment is necessary before a final report on the test result is presented. Results can be expressed as the percentage infected or contaminated seeds, the number of fungal spores or colony-forming units per gram or number of seeds tested, the occurrence or non-occurrence of the pathogen in a working sample or number of subsamples, etc. In the latter case MPN tables can be used to estimate the number of infested seeds by the most probable number method (Taylor & Phelps, 1984). For official seed health testing, it is usually necessary to state the maximum infection level for a negative result at a given probability (e.g. $P = 0.05$). It is also important to note in a test report the size of the working sample i.e. the number of seeds tested, and the method of statistical analysis.

Incorrect, improper, illusory analytical data

Certain methods, especially those used for detection of low infection levels of bacteria or viruses may sometimes give false positive and false negative values. Examples of how to interpret these have been worked out by Sheppard *et al.* (1986).

DISCUSSION

High quality standards are required for the successful marketing of vegetable seeds worldwide. An important element of quality is the health status, the foundation of which is established during the seed production but may be improved subsequently by technological processes. Diagnostic methods by which the presence of plant pathogens in seed can be detected are mostly used at the stage when seed lots are ready for sale. There are several other stages at which application of such methods would be justified, thereby contributing to more efficient quality control management between the time the seed is produced and used. Figure 1 and the comments made on the possible reasons for testing, make it clear that obtaining health data before harvest can be used for planning and organising the technological processes which follow after harvest. Data obtained during the various processing stages will help to avoid unnecessary spoilage of seed through cross contamination between and within seed lots. Moreover, such data could also be used to choose the best seed treatment if needed.

Another aspect requiring comment concerns the kind of method to be chosen at a particular stage of processing. It has been emphasized that the choice of method depends on the purpose of testing, the nature of the pathogen and the principle of the method. A great diversity of methods is available currently and new ones are still being developed, mostly based on molecular techniques. Nevertheless, it has been pointed out that special attention should be given to the interpretation of test results obtained by the modern methods as they often do not give information about the viability of the inoculum detected. The latter aspect is important if the target organism is not considered as a quarantine pathogen and a certain level of contamination can be tolerated. Very sensitive, specific and fast methods may be required to detect the survival and presence of living inoculum of the pathogen after a seed treatment, provided the aim of the treatment was to kill the inoculum or to reduce the transmission rate to acceptable levels.

Tests may give false negative or false positive results (Sheppard *et al.*, 1986). For example, heavy contamination of seeds with saprophytic fungi or bacteria may mask the presence of a pathogen when plating the seed or seed extracts on filterpaper or nutrient agar (de Tempe & Limonard, 1973; Franken *et al.*, 1991). Also, seeds of different cultivars may respond in different ways to the various treatments. Furthermore, residues of chemicals on the seed, applied either on the seed crop or after harvest during any stage of the processing, may also interfere with the result of the seed health test (Franken *et al.*, 1993).

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REFERENCES

- Agarwal, V.K.; Sinclair, J.B. (1987) *Detection of seedborne pathogens, Principles of seed pathology, volume II*, CRC Press, Inc, Boca Raton, Florida, USA, 29-76.

- Anonymous (1989) Guidelines for collaborative studies, *Journal of the Association of Analytical Chemists*, 72, 694-704.
- Anonymous (1993) Seed Health Testing in: Annexes to chapter 7 of International Rules for Seed Testing, 1993, *Seed Science and Technology*, vol. 21, supplement, 211-216.
- Franken, A.A.J.M.; van Zeijl, C.; van Bilsen, J.G.P.M.; Neuvel, A.; de Vogel, R.; van Wingerden, Y.; Birnbaum, Y.E.; van Hateren, J.; van der Zouwen, P.S. (1991) Evaluation of a plating assay for *Xanthomonas campestris* pv. *campestris*, *Seed Science and Technology*, 19, 215-226.
- Franken, A.A.J.M.; Kamminga, G.C.; Snijders, W.; van der Zouwen, P.S.; Birnbaum, Y.E. (1993) Detection of *Clavibacter michiganensis* ssp. *michiganensis* in tomato seeds by immunofluorescence microscopy and dilution plating, *Netherlands Journal of Plant Pathology* 99, 125-137.
- Geng, S.; Campbell, R.N.; Carter, M.; Mill, F.J. (1983) Quality control programs for seedborne pathogens, *Plant Disease* 67, 236-242.
- Hampton, R.; Ball, E.; De Boer, S. (1990) *Serological methods for detection and identification of viral and bacterial plant pathogens. A laboratory manual*, APS Press, the American Phytopathological society, St. Paul, Minnesota, USA, 389 pp.
- International Organization for Standardization (1985) *ISO 7218, Microbiology General guidance for microbiological examinations*, International organization for standardization, Geneva, Switzerland, 14 pp.
- International Seed Testing Association (1981 -) *ISTA Handbook on seed health testing, section 2, Working sheets, each dealing with one pathogen on one host*, Zürich, Switzerland.
- International Seed Testing Association (1993) *International Rules of Seed Testing 1993, Seed Science and Technology* 21, Supplement, Zürich, Switzerland, 288 pp.
- Langerak, C.J.; Merca S.D.; Mew, T.W. (1988) Facilities for seed health testing and research, *Proceedings of the International Workshop on Rice Seed Health*, 16-20 March, 1987, pp 235-246.
- Middleton, J.T. (1944) Seed transmission of squash mosaic virus, *Phytopathology*, 34, 405.
- Mujica, F. (1943) La sepioriosis del Apio. *Simiente*, 12, 81.
- Neergaard, P. (1977) *Seed Pathology, Volume I and II*, The MacMillan Press LTD, London, England, 1187 pp.
- Richardson, M.J. (1990) *An annotated list of seed-borne diseases, Section 1.1., ISTA Handbook on Seed Health Testing*, International Seed Testing Association, Zürich, Switzerland.
- Schaad, N.W. (1988) *Laboratory guide for identification of plant pathogenic bacteria*, American Phytopathological Society, St. Paul, Minnesota, USA.
- Saettler, A.W.; Schaad, N.W.; Roth, D.A. (1989) *Detection of bacteria in seed and other planting material*, 122 pp., APS Press, The American Phytopathological Society, St. Paul, Minnesota, USA.
- Sheppard, J.W.; Wright, P.F.; DeSavigny, D.H. (1986) Methods for the evaluation of EIA tests for use in the detection of seed-borne diseases, *Seed Science and Technology*, 14, 49-59.
- Taylor, J.D.; Phelps, K. (1984), Estimation of percentage seed infection, *Report on the first International Workshop on Seed Bacteriology*, Angers, France, 12-14.
- Tempe de, J.; Limonard, L. (1973) Seed-fungal-bacterial interactions, *Seed Science and Technology*, 1, 203-204.

SEED-BORNE PATHOGENS OF LINSEED IN THE UK

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ABSTRACT

The commonest seed-borne pathogens of linseed in the UK are *Alternaria linicola*, *Botrytis cinerea*, and *Fusarium* spp. Their incidence is dependent on a number of factors, the most important of which is the weather. Most of the pathogens are found only on the outside of the seed and do not infect the embryo; they are thus relatively easily controlled by seed-treatments at the seedling stage. However, some pathogens, particularly *B. cinerea*, may also be transmitted in other ways and can attack the growing crop independent of their incidence on the seed and may require further control measures. Choice of cultivar may also influence the incidence of seed-borne disease.

INTRODUCTION

Linseed in the UK suffers from a number of diseases which can be seed-borne (Mercer *et al.*, 1991). The damage caused to the emerging seedling is dependent on a combination of the incidence of the pathogens on the seed and the weather conditions at the time of sowing (Fitt *et al.*, 1991). Although little can be done about the latter factor, the incidence of seed-borne pathogens can be reduced by the judicious choice of seed or by seed-treatment. This paper describes the background to current practices and suggests ways that they could be improved in the future.

PATHOGENS

Those pathogens most commonly isolated from linseed seed in the UK (Fig. 1) are *Alternaria linicola*, *Botrytis cinerea* and *Fusarium* spp. (mostly *F. avenaceum* and *F. culmorum*). Others less frequently isolated are *Phoma exigua* var. *linicola*, *Colletotrichum linicola*, *Mycosphaerella lini* and *Fusarium oxysporum* f.sp. *lini*. The incidence of pathogens is largely determined by the weather during the period of capsule maturation, being considerably higher if this is wet as in 1987/88 (Fig. 1). The effect of weather is also reflected in regional differences in pathogen incidence, *A. linicola*, for example, being readily isolated from seed every year in Northern Ireland, while sometimes being at a relatively low incidence in the drier south east of England. Conversely, *F. oxysporum* f.sp. *lini*, which requires relatively high soil temperatures, is a major problem on the continent, is occasionally found in the

south east of England, but has not been recorded in Northern Ireland in recent years.

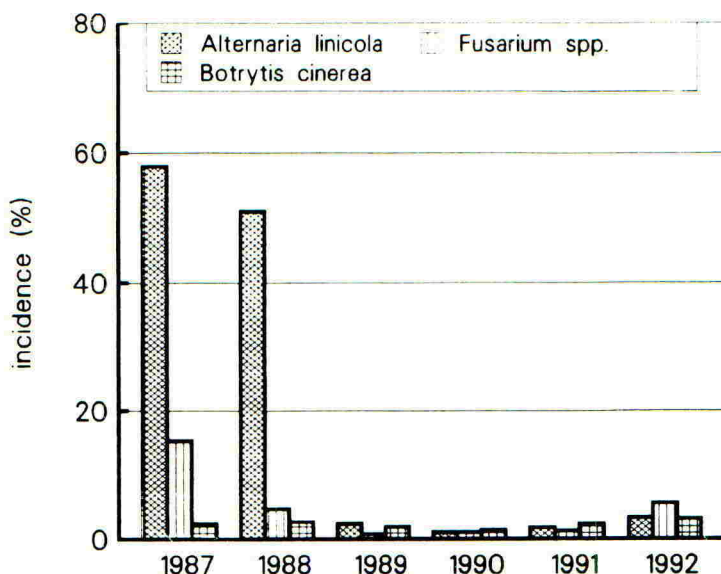


FIGURE 1. Mean percentage incidence (when present) of seed-borne pathogens on UK linseed seed from 1987 - 1992.

EPIDEMIOLOGY

Pathogen transmission

Although the pathogens above have been classified as seed-borne, many of them may also be transmitted in other ways. *Botrytis cinerea* is a ubiquitous soil- and air-borne pathogen and can infect the growing linseed crop each year independent of the health-status of the seed, if weather conditions are suitable (Mercer *et al.*, 1991). Similarly, *Fusarium* spp. have been readily isolated in N. Ireland from lesions on roots of linseed plants grown in the field from seed free of pathogens (P.C. Mercer, unpublished). *Phoma exigua* var *linicola* has been observed to cause severe damage in experimental plots in N. Ireland (Mercer & Hardwick, 1993a) even though there was no indication of its presence on the seed (P.C. Mercer, unpublished). There was also evidence from a trial in N. Ireland in 1993 of a low, but significant level of transmission of *A. linicola* via the soil (P.C. Mercer, unpublished), even though it is clear that the main method of transmission is via the seed.

Position of pathogens on the seed

Most of the important pathogens of linseed capable of being seed-borne in the UK, are found in the seed coat (Mercer & Hardwick, 1991) where they appear to be present as resting hyphae. There is little evidence for the presence of spores or other propagules. Nor is there much evidence for fungal colonisation of the embryo. Ultrastructural studies have shown that the resting hyphae are located mainly in cells underlying the outermost gelatinous layer (P.C. Mercer, unpublished). From here they can rapidly resume growth, as the seed imbibes water, and grow out to infect the erstwhile sterile seedling.

Host/pathogen interaction at germination

The degree to which the pathogen is successful at colonising the seedling is dependent on weather conditions at the time of sowing. Low temperatures favour the pathogens at the expense of the host, while at higher temperatures, the host is able to grow sufficiently vigorously to "escape" what are generally relatively weak pathogens. An experiment carried out in a heated and an unheated glasshouse in N. Ireland with samples of seed with different levels of *Alternaria linicola* showed a higher incidence of the pathogen on the roots of seedlings germinated in the unheated glasshouse compared with the heated (Fig. 2). Percentage germination was also significantly poorer in the unheated house, although in this instance it was not significantly correlated with the percentage

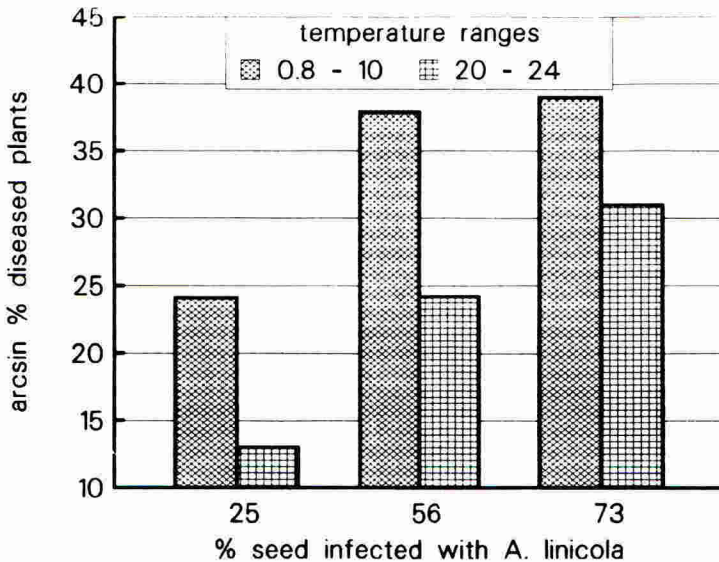


FIGURE 2. Effect of temperature at germination and percentage of seed infected with *Alternaria linicola* on the incidence of *A. linicola* on roots of 15 cm high seedlings.

incidence of *A. linicola* on the seed. However, this can occur under field conditions - in a trial in N. Ireland in 1988 there was a reduction in emergence of at least 50% resulting from using seed with an incidence of 57% *A. linicola* (Mercer & Hardwick, 1991). This led to a consequent drop in yield of 15%.

Effect of seed-health on the growing crop

Although much of the effect of seed-borne pathogens is observed at crop emergence, the growing crop is also subject to attack from a range of pathogens, some of which are also capable of being seed-borne, e.g. *Botrytis cinerea*, *Fusarium* spp. and *Phoma exigua* var. *linicola*. There generally appears to be little correlation between incidence of the pathogen in the seed and incidence in the growing crop. However, disease-assessment of a linseed trial at growth stage 50 (Freer, 1991) in N. Ireland in 1993 (P.C. Mercer, unpublished) showed that the incidence of *Alternaria linicola* on untreated seed could be correlated positively with its later incidence on roots, stem-bases and leaves, although not capsules (Fig. 3). An assessment one week later indicated that there might even be a slight correlation with capsule-colonisation.

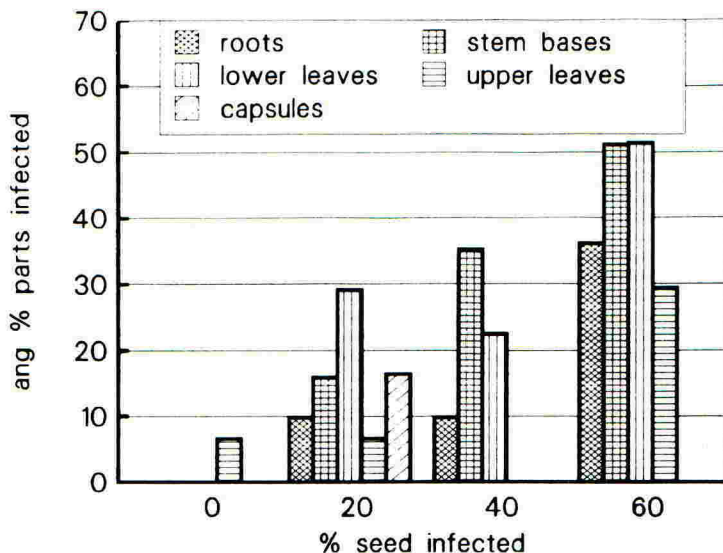


FIGURE 3. Effect of incidence of *Alternaria linicola* on seed of linseed on its later isolation from other plant parts (GS 50, N. Ireland, 1993).

These correlations may result from the observed colonisation pattern of this pathogen, moving from lower to upper plant parts with time (Fig. 4) but without large numbers of spores being produced until the end of capsule production (Mercer et al., 1992).

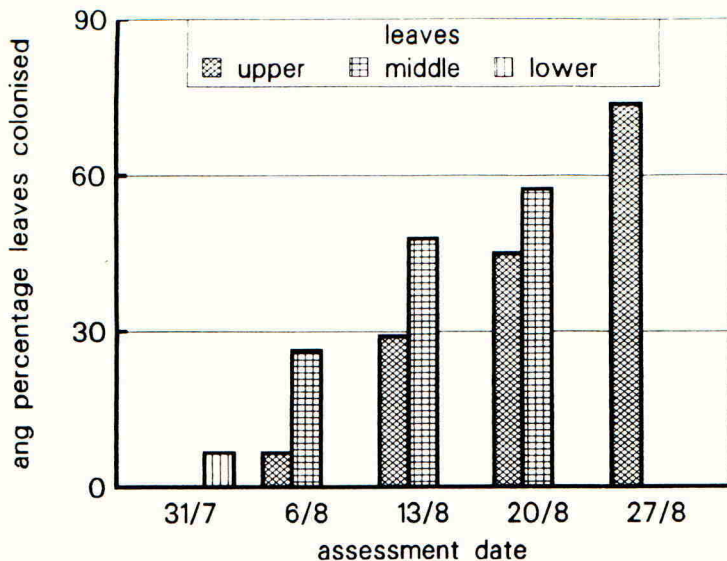


FIGURE 4. Progressive colonisation of leaves by *Alternaria linicola* on linseed plants in N. Ireland in 1991.

Infection of seed by pathogens

Seed-borne pathogens enter capsules and seeds as these organs mature. Passage appears to be either through the capsule's walls or central stalk. The means whereby the actively growing pathogen becomes converted into resting hyphae embedded in the seed's gelatinous layer is largely unknown.

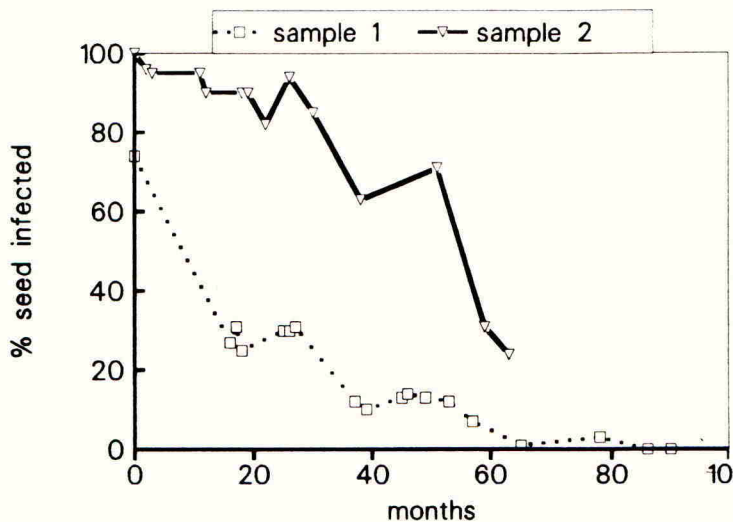


FIGURE 5. Effect of time on *Alternaria linicola* viability in seed (initial infections of samples: 1 - 74%; 2 - 100%).

Viability of pathogens on seed

Viability of resting hyphae in the seed depends strongly on the pathogen. The incidence of *Botrytis cinerea* and *Fusarium* spp. declines rapidly over a few months after harvest (Mercer *et al.*, 1991), while hyphae of *Alternaria linicola* can remain alive for at least five years (Fig. 5).

CONTROL

Seed-treatments

The most effective method of control of damage to seedlings by seed-borne pathogens is the use of a seed-treatment. In the early 1980s, the small amount of linseed seed being sown was generally treated with a mixture of benomyl and thiram. However, research indicated that an iprodione powder formulation (Rovral, Rhone-Poulenc Ltd.) could give complete control of *Alternaria linicola* and improved percentage germination (Mercer *et al.*, 1985). This product then became the industry standard.

However, at that point *A. linicola* was perceived as the most important seed-borne pathogen and research was concentrated on its control. In 1985, some seed had, as well as *A. linicola*, a relatively high incidence of *Fusarium avenaceum*, which because of its suppression by *A. linicola*, frequently only became apparent following application of iprodione (which controlled *A. linicola* only). Control of the *F. avenaceum* required the further addition of benomyl (Mercer & McGimpsey, 1987).

Resistance by *A. linicola* to iprodione appeared first in 1986 and spread rapidly so that by 1988, 85% of seed samples had at least some of their *A. linicola* population resistant to iprodione (Mercer *et al.*, 1991). An intensive search was then made for alternative products and thirty-four were examined (Mercer & Hardwick, 1993b). Some products such as fenpropimorph/benomyl and propiconazole/tridemorph showed good control of both *A. linicola* and *F. avenaceum*, but had an inhibiting effect on germination. The most effective and least damaging fungicides were propiconazole and prochloraz, the latter being more effective in the control of *A. linicola* (Mercer *et al.*, 1988). At present a prochloraz formulation (Prelude, Schering plc) is the most widely used seed-treatment on UK linseed.

However, examination of *A. linicola* populations on random samples of seed in 1993 (P.C. Mercer, unpublished) showed no further indications of resistance to iprodione. It is probable that resistant strains of *A. linicola* are less fit than sensitive ones and that the considerably reduced usage of iprodione has allowed the sensitive strains to re-establish. A comparison between prochloraz and iprodione in 1993 showed similar improvements in emergence and reductions in *A. linicola* over an untreated control (Fig. 6).

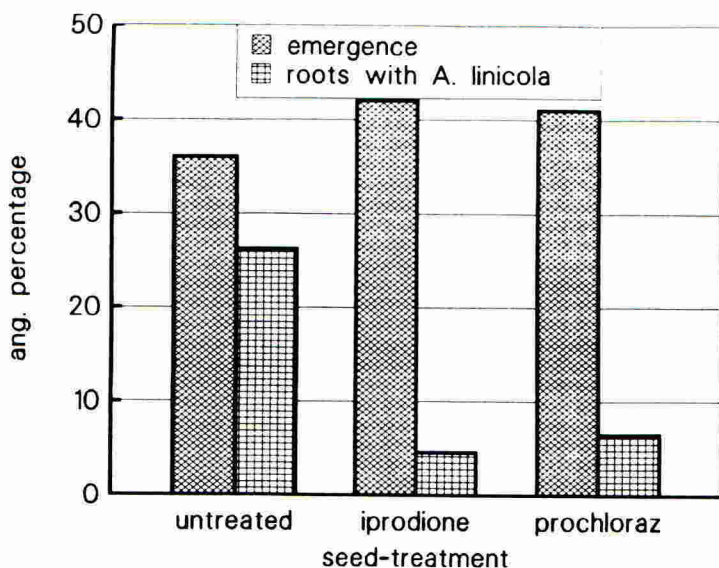


FIGURE 6. Effect of seed-treatment on emergence and root infection (GS 15) by *Alternaria linicola* following the use of seed which had an incidence of 60% *A. linicola* (N. Ireland, 1993).

Crop spraying to reduce seed-borne disease

The application of fungicide sprays to the growing crop has been shown to reduce the incidence of some seed-borne pathogens, e.g. *A. linicola* (Fitt & Ferguson, 1990; Mercer & Ruddock, 1993) and *B. cinerea* (Mercer & Hardwick, 1991). However, the reduction in incidence of *A. linicola* is variable and frequently not below the 5% level required for seed-certification. Further, although fungicide sprays do reduce *B. cinerea* on the growing crop and can increase yields, the tendency for badly infected capsules to fall to the ground may sometimes result in a negative relationship between the incidence on the crop and on the seed (Thomas et al., 1993). The economics of linseed, at present, will only allow for a single fungicide spray and it is not yet clear when this is most effectively applied (Mercer & Hardwick, 1991).

Use of cultivars to control seed-borne disease

Some cultivars are more resistant to some of the seed-borne diseases than others. For example, the incidence of *A. linicola* on the capsules and seeds of cv. Andro was much lower than that on other cultivars in a trial in N. Ireland in 1992 (Mercer & Ruddock 1993). There are also indications of cultivar effects in susceptibility to *B. cinerea* (Thomas et al., 1993), although, as observed above, incidence on the growing crop does not always correlate with that on the seed.

Although the link between cultivar maturity and susceptibility to seed-borne diseases is not very clear, it does appear that crops which are harvested late with wet conditions prior to harvest are more prone to seed-borne disease (Fig. 1). It would therefore be expected that those cultivars requiring only a short growing season (and perhaps autumn-sown cultivars) would have less seed-borne disease.

CONCLUSIONS AND FUTURE DEVELOPMENTS

The location of most seed-borne pathogens only in the seed coat makes them ideal candidates for control by seed-treatment. Although present treatment is generally highly effective, it would be preferable if a wider range of products were available to obviate any possible build-up in fungicide-resistance. Control by seed-treatment of pathogens which can be seed-borne does not necessarily rule out their later presence on the growing crop. This is particularly so of *Botrytis cinerea* which may require further control measures.

In the future, varietal control may be more widely used for disease control in linseed, and biocontrol seed-treatments may also become available. However, a major requirement for the evolution of the most effective disease-control measures is a much fuller understanding of the epidemiology of the seed-borne pathogens.

REFERENCES

- Fitt, B.D.L. & Ferguson, A.W. (1990) Responses to pathogen and pest control in linseed. *Brighton Crop Protection Conference - Pests and Diseases 1990*, 2, 733-738.
- Fitt, B.D.L., Ferguson, A.W., Dhua, U. & Burhenne, S. (1991) Epidemiology of *Alternaria* spp. on linseed. *Aspects of Applied Biology, Proceedings of Conference on Production and Protection of Linseed*, 28, 95-100.
- Freer, J.B.S. (1991) A development stage key for linseed (*Linum usitatissimum*). *Aspects of Applied Biology, Proceedings of Conference on Production and Protection of Linseed*, 28, 33-40.
- Mercer, P.C. & Hardwick, N.V. (1991) Control of seed-borne diseases of linseed. *Aspects of Applied Biology, Proceedings of Conference on Production and Protection of Linseed*, 28, 71-78.
- Mercer, P.C. & Hardwick, N.V. (1993a) Methods of control of diseases of linseed. *Proceedings of Crop Protection in Northern Britain 1993*, 165-170.
- Mercer, P.C. & Hardwick, N.V. (1993b) Chemical control of seed-borne diseases of linseed in the UK. *Proceedings of the International Organisation for Biological Control (West Palearctic Region) Rennes, France 1992* (in press).
- Mercer, P.C. & McGimpsey, H.C. (1987) Joint control of *Alternaria linicola* and *Fusarium avenaceum* on the seed of linseed. *Tests of Agrochemicals and Cultivars (Annals of Applied Biology* 110, Supplement) 8, 54-55.

- Mercer, P.C. & Ruddock, A. (1993) Effects of cultivars and iprodione on the incidence of *Alternaria linicola* in capsules and seeds of linseed in Northern Ireland. *Tests of Agrochemicals and Cultivars (Annals of Applied Biology 122, Supplement) 14*, 146-147.
- Mercer, P.C., McGimpsey, H.C. & Ruddock, A. (1988) The control of seed-borne pathogens of linseed by seed-treatments. *Tests of Agrochemicals and Cultivars (Annals of Applied Biology 112, Supplement) 9*, 30-31.
- Mercer, P.C., Hardwick, N.V., Fitt, B.D.L & Sweet, J.B. (1991) Status of diseases of linseed in the UK. *Home-Grown Cereals Authority Research Review OS3*, 76 pp.
- Mercer, P.C., McGimpsey, H.C., Black, R. & Norrie, S. (1985) The chemical control of *Alternaria linicola* on the seed of linseed. *Tests of Agrochemicals and Cultivars (Annals of Applied Biology 106, Supplement) 6*, 56-57.
- Mercer, P.C., Ruddock, A., Fitt, B.D.L & Harold, J.F.S. (1992) Linseed diseases in the UK and their control. *Brighton Crop Protection Conference - Pests and Diseases 1992*, 3, 921-930.
- Thomas, J.E., Mercer, P.C. & Ruddock, A. (1993) Incidence of *Botrytis cinerea* on linseed cultivars at sites in England and Northern Ireland. *Tests of Agrochemicals and Cultivars (Annals of Applied Biology 122, Supplement) 14*, 150-151.

MERCURY-BASED SEED TREATMENTS FOR CONTROL OF BACTERIAL BLIGHT IN COTTON

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ABSTRACT

The evaluation of mercury based compounds in Nigeria for bacterial blight control commenced in 1953. Disease incidence, in some of the trials, were reduced by as much as 93%. Formulations of a mixture of phenyl mercuric acetate (PMA) and ethyl mercuric chloride (EMC) (3% or 5% Hg), were then recommended for treating all cottonseed used by farmers. However, these mercurials exhibited high mammalian toxicity and phytotoxicity. Cuprous oxide (45% Cu), a less effective bactericide, was also recommended. Based on trials conducted between 1967 and 1970, bronopol (12%), a less poisonous and effective bactericide, was recommended as an alternative to the mercurial and copper formulations. Recent experiments have found liquid formulations containing 30% or 60% of 2-(thiocyanomethylthio)-benzothiazole (TCMTB) and acid-treatment to be as effective as bronopol in disease control. The problems that were associated with the use of mercury based seed treatments by Nigerian cotton farmers are discussed.

INTRODUCTION

Bacterial blight (*Xanthomonas campestris* pv. *malvacearum*) is the major disease of cotton in Nigeria. Annual yield loss is estimated at 10 -20% (Dransfield, 1965). Integrated management strategy for the disease involves the combined use of field sanitation, partially resistant cultivars and chemical seed treatment. The evaluation of seed treatment chemicals commenced in 1953 at the now Institute for Agricultural Research, Samaru, with particular attention being paid to their efficacy against bacterial blight, cost effectiveness and levels of both mammalian toxicity and phytotoxicity (Dransfield, 1968). This paper reports the contributions of mercury based seed treatment chemicals and their alternatives in bacterial blight control and cotton production in Nigeria and highlights some of the socio-economic and technical problems associated with their use.

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MATERIALS AND METHODS

Over 60 different seed treatment chemicals were evaluated between 1953 and 1967 (Dransfield, 1968). Organo-mercurial dust formulations tested included: PMA - phenyl mercuric acetate + EMC - ethyl mercuric chloride (Agrosan 3W, 3% Hg), PMA + EMC (Agrosan 5W, 5% Hg), MC - mercuric chloride + MI - mercuric iodide (Abavit B, 8.4% Hg), PMU - phenyl mercury urea (Abavit B red, 1.0% Hg), M - mercury (1.25%) + D - dieldrin (75%) (Dieldrex A (1.25% Hg), PMA (Leytosan P, 3.0 - 7.6% Hg), MMS - methoxyethyl mercuric silicate (Leytosan M, 3.0 - 7.6% Hg) and EMC + PMA (Leytosan E, 3.0 - 7.6% Hg). The three organo-mercurial liquid formulations assessed were MMN - methyl mercury nitrile (Agrosol, 1.0% Hg), CMG - cyanomethyl - mercury - guanidine (Panogen, 1.5% Hg) and PMAA - phenyl mercuric ammonium acetate (Mist-O-Matic, 4% Hg). Copper formulations tested were cuprous oxide (Shell Cu_2O , 50% Cu) and cuprous oxide (Cuprocot, 45% Cu). The dust and liquid mercury formulations were applied at a dosage rate (weight of chemical: weight of seed) varying from 1: 100 - 1: 400. The dosage rate of the copper formulations ranged from 1: 100 - 1: 300. Machine delinted (fuzzy) Samaru 26C seed was used in 1953, and Samaru 26 J seed subsequently. To ensure proper seed coating, seeds and chemicals were vigorously shaken in closed containers for 5 minutes. Replicated field trials were usually sown (June or July) in randomized block designs (Dransfield, 1968). Plots consisted of one, two or three ridges (10m x 0.9m). Six seeds of each treatment were sown per hill/stand, using a within-row spacing of between 15 - 45 cm. Parameters assessed for each treatment were stand counts, seedling emergence, and bacterial blight incidence. Proportional reduction in bacterial blight - a measure of the efficiency of each treatment - was expressed as the mean ratio of the percent diseased plants in treated plots to that of the untreated plots.

Trials between 1967 and 1972 were conducted with non-mercurial seed treatment chemicals. Two new dust formulations, Bronocot (12% bronopol) and Unicot (40% cufraneb), were compared with standard recommended seed treatment chemicals, PMA + EMC (3% Hg) and cuprous oxide (45% Cu), based on the 1953 - 1967 experiments (Dransfield & Beeden, 1974). The new chemicals were evaluated at a dosage rate of between 1: 100 and 1: 250 w/w. Seeds of Samaru 26J were used. Experimental layouts were generally similar to those of the 1953 - 67 trials. However, yield trials were conducted on larger plots (up to 200m²). Parameters evaluated were germination, disease incidence and severity, and yield of seedcotton.

Over ten non-mercurial liquid and dust/powder chemicals were compared with bronopol in laboratory, glasshouse and field tests, between 1980 and 1989 (Poswal & Erinle, 1987; Poswal *et al.*, 1992). The effect of acid-delinted seeds, with or without chemical seed treatment, was also evaluated. Machine - and acid-delinted seeds of Samaru 71, Samaru 72 and Samaru 77 were used. All new chemicals were tested at the manufacturers recommended rates. Liquid formulations were applied, as slurries, by firstly dispersing the recommended rates in quantities of water equivalent to 2.0% of the weight of seed treated. Cotton seeds and the chemicals were placed in polythene bags and agitated until the seed was uniformly coated. The procedure of Cross (1962) for acid-treatment of machine-delinted seed was used. Seed-seedling parameters evaluated in the laboratory and glasshouse tests were germination and seedling emergence, radicle damage, root length and seedling height. Seedling emergence, disease incidence and severity, and yield of seedcotton were parameters assessed in the field.

RESULTS AND DISCUSSION

The relative efficacy of some of the over 60 seed treatment chemicals tested between 1953 and 1967 is presented in Table 1. The dust mercurials, represented by the PMA + EMC, PMA and MMS formulations were the most effective, compared to the non-mercurials (cuprous oxide). A reduction in the application rates from 1: 100 to 1: 400 lead to reduced bacterial blight control. PMA + EMC (5% Hg) at the rate of 1: 150, was adopted as the standard commercial treatment until 1966 (Dransfield, 1968).

TABLE 1. Summary of the relative efficiency of some of the seed treatment chemicals evaluated between 1953 and 1967.

Seed treatment	Dosage rate	Rank order	Percentage* efficiency
PMA + EMC (5% Hg)	1 : 150	1	96.4
	1 : 200	3	96.0
	1 : 250	32	86.8
	1 : 300	30	87.6
PMA + EMC (3% Hg)	1 : 150	12	93.7
	1 : 250	43	73.3
PMA	1 : 150	5	95.6
MMS	1 : 150	6	95.2
EMC + PMA	1 : 150	13	93.1
M + D (1.25% Hg)	1 : 150	26	89.2
	1 : 100	4	95.8
CMG (1.5% Hg)	1 : 200	15	91.9
	1 : 300/400	33	86.3
	1 : 100	11	94.2
PMAA (4% Hg)	1 : 200	21	90.4
	1 : 300/400	40	79.6
	1 : 150	38	80.5
Cuprous oxide (45% Cu)	1 : 200	46	66.4
	1 : 150	45	69.2
Cuprous oxide (50% Cu)	1 : 250	56	48.7

* Percentage efficiency = $100 - (\text{Mean ratio} \times 100)$; Mean ratio = mean of ratio of percent diseased plants in treated to untreated plots).
Source: Dransfield (1968)

In 1966 the rate of application of PMA + EMC (5% Hg) was reduced to 1: 200, and disease incidence varied from 2.7% to 4.8% and from 96.7% to 100% for the untreated control. By 1967, PMA + EMC (3% Hg) (1: 150) replaced PMA + EMC (5% Hg) (1: 200) due to the comparable level of disease control recorded between 1965 and 1967. The PMA + EMC (3% Hg) dosage rate was further reduced to 1: 170. None of the liquid mercurial treatments were recommended for commercial use. This was due to the high degree of phytotoxicity expressed by MMN (1.0% Hg) and CMG (1.5% Hg). No phytotoxic effects were observed with PMAA or with PMA + EMC formulations. In addition, machines for treating liquid treatments became clogged and choked by damp lint. Consequently the objective of testing liquid treatments as alternatives to the dust chemicals, given their health hazards during the seed ginning and treatment process, was abandoned. Cuprous oxide (45% Cu), a less efficient but safer treatment, was also recommended.

By 1966, copper treatments were increasing in cost and the disadvantage of organo-mercurial treatments became widely known and a matter of concern to the Nigerian government. Intensive research between 1967 and 1970 with substitute formulations, containing other active ingredients, lead to the recommendation of bronopol. Differences between bronopol and PMA + EMC (3% Hg) were not significant; however, PMA + EMC (3% Hg) consistently gave higher seed germination and lower disease incidence and severity (Table 2). Similarly, increase in seedcotton yields, over the control, were 10.5%, 11.7% and 10.2% for PMA + EMC (3% Hg), cuprous oxide (45% Cu) and bronopol, respectively (Table 3). In five year trials, (Dransfield & Beeden, 1974) showed that cufraneb (40%) at a dosage rate of 1: 150 was as effective as the mercurial and bronopol formulations, and more effective than the copper treatments (Table 4). Furthermore, no evidence of phytotoxicity was indicated. As a result of its low mammalian toxicity (acute oral LD₅₀ = 2700 mg/kg) compared to those of PMA + EMC (3% Hg) (30 - 50 mg/kg) and bronopol (400 mg/kg), cufraneb was added to the list of recommended seed treatments.

TABLE 2. Bacterial blight in large-scale trials at Samaru in 1968-70

Seed treatment	Dosage rate (wt/wt)	Germination (%)	Disease incidence (%)	Mean disease score (0-5 Scale)
PMA + EMC (3% Hg)	1 : 150	67	0.7	0.01
Bronopol	1 : 150	59	1.3	0.03
"	1 : 160	59	2.2	0.05
"	1 : 170	60	2.5	0.05
"	1 : 180	59	3.0	0.06
"	1 : 190	58	3.9	0.09
"	1 : 200	57	4.5	0.10
"	1 : 225	60	6.9	0.17
"	1 : 250	60	8.2	0.19
Untreated control	-	61	69.2	2.05

Source: Dransfield (1971)

Poswal & Erinle (1987) highlighted certain problems associated with the practice of producing and distributing treated cotton seed in Nigeria. The use of dust chemicals consistently creates potential health hazards, while poor storage and handling of treated machine-delinted seed may also lead to excessive loss of the chemicals before planting. As a result of the bulk packaging (in 25 - 50kg jute or polypropylene bags), there is frequent wastage and misuse of treated seed. Most disturbing, however, is the practice of washing and feeding Bronocot treated seeds to livestock by many farmers. The defunct Nigerian Cotton Board then began investigations into the feasibility of replacing the old plantector and drum ginning machines with mechanical and slurry seed treaters. Consequently, the evaluation of non-mercurial liquid bactericides, Busan 30 and Busan 72, containing 30% and 60% of TCMTB, respectively, was initiated in 1980. When compared to bronopol, no significant differences were recorded (Table 5). This preliminary trial suggested that TCMTB (30%) is a potentially good alternative to bronopol (Poswal & Erinle, 1987).

TABLE 3. Yields of seedcotton in 1968 and 1969 trials (sown mid- June and sprayed against insects), combined over five locations.

Seed treatment	Yields (kg/ha)	
	Mean	% increase over control
12% Bronopol	985	10.2
45% Copper	999	11.7
3% Mercury	988	10.5
Control	894	-

Source: Dransfield (1971)

TABLE 4. Relative efficiency of cufraneb for bacterial blight control at Samaru in 1972.

Seed treatment	Germination (%)		Disease incidence (%)	Mean disease score (0-5 scale)
	7 days	13 days		
PMA + EMC (3% Hg)	71	98	3.4	0.06
Cufraneb	80	97	1.8	0.04
Bronopol	72	98	3.5	0.08
Untreated	77	98	65.3	2.32
S.E ±	6.4	1.2	2.44	0.051

Source: Dransfield & Beeden (1974)

Laboratory, glasshouse and field trials with non-treated and chemically treated acid-delinted seed lots gave significantly higher seed germination and seedling emergence, and more vigorous seedlings than machine-delinted seeds. The incidence and severity of bacterial blight was also reduced and seed cotton yields increased (Table 6).

TABLE 5. The effect of TCMTB (3%) and TCMTB (60%) on the development of bacterial blight in 1980.

Bactericidal treatment	Dosage rate (A.I.kg/seed)	Germination (%)	Disease incidence (%)	Disease severity (0-5 scale)
TCMTB (60%)	9.3 ml	58.2	23.2	0.42
TCMTB (30%)	18.0 ml	58.5	19.4	0.30
Bronopol	20.0 g	59.6	17.8	0.29
Untreated	-	53.1	74.1	1.80
L.S.D. (0.05)		N.S.	6.84	0.16

Poswal & Erinle (1987)

It is hoped that cotton production in Nigeria could be enhanced if farmers are issued with acid-delinted and chemically treated seeds in small polythene or polypropylene bags, commensurate with their yearly requirements. This should eliminate some of the problems and misuse associated with machine-delinted seed (Poswal *et al.*, 1992).

TABLE 6. Effect of delinting method on bacterial blight incidence/severity, seedling emergence and seedcotton yield.

Delinting method	Bacterial blight		Seedling emergence (%)	Seedcotton yield (kg/ha)
	Incidence (%)	Severity (0-5 scale)		
Machine	86.3	1.86	42.3	1347
Acid	51.8	0.84	64.0	1656

Source: Poswal (1987)

REFERENCES

- Cross, J.E. (1962). An improved method of acid-treating small quantities of cotton seed. *Empire Cotton Growing Review*, 39, 205.
- Dransfield, M. (1965). Cotton seed dressing in northern Nigeria. *Samaru Research Bulletin*, 48, 261-265.
- Dransfield, M. (1968). Seed dressing trials on cotton in northern Nigeria, 1953-1967. *Samaru Miscellaneous Paper*, 26, 1-22.
- Dransfield, M. (1971). Seed dressing trials to control bacterial blight of cotton (*Xanthomonas malvacearum*) in the northern States of Nigeria. *Proceedings of the Sixth British Insecticide and Fungicide Conference*, 225-230.
- Dransfield, M.; Beeden, P. (1974). A new cotton dressing for northern Nigeria. *Samaru Miscellaneous Paper*, 46, 1-9.
- Poswal, M.A.T. (1987). Survival of *Xanthomonas campestris* pv. *malveacarum* and performance of seedlings from commercially ginned cottonseed as influenced by length of storage. In: *Plant Pathogenic Bacteria*, E.L. Civerolo, A. Collmer, R.E. Davis and A.G. Gillapsie (Eds), Netherlands: Nijhoff Publishers, pp. 741-745.
- Poswal, M.A.T.; Erinle, I.D. (1987). Comparative study of the efficacy of Busan 30, Busan 72 and Bronocot for the control of bacterial blight of cotton in northern Nigeria. In: *Plant Pathogenic Bacteria*, E.L. Civerolo, A. Collmer, R.E. Davis and A.G. Gillapsie (Eds), Netherlands: Nijhoff Publishers, pp. 976-981.
- Poswal, M.A.T.; Atangs, P.A.; Akpa, A.D. (1992). Laboratory and glasshouse evaluation of seed treatment chemicals in relation to some seed-seedling parameters in cotton. *Seed Science and Technology*, 20, 69-76.

FILM-COATING OF LEEK SEEDS WITH INSECTICIDES: EFFECTS ON GERMINATION AND ON THE CONTROL OF ONION FLY (*DELIA ANTIQUA* (MEIGEN))

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ABSTRACT

Field experiments were carried out in 1991 and 1992 to assess the control of the onion fly (*Delia antiqua*) in a winter leek crop (*Allium porrum* L.) by film-coating the seeds with insecticides. Plants were raised in a seed-bed.

The efficacy of benfuracarb, carbofuran, imidacloprid and isofenphos at two rates as seed film-coatings was compared to a conventional application of chloorfenvinphos to the seed-bed plus two spray applications with carbofuran. Germination of only benfuracarb film-coated seed was comparable to the untreated controls in all tests. Control of onion fly by benfuracarb applied as a filmcoating was as effective as the conventional application.

This seed treatment will reduce the necessary amount of insecticide for control of the onion fly in a winter leek crop by 98% to 3 g per 100 m² seed-bed.

INTRODUCTION

Increasing costs and increasing concern about the environmental impact of insecticides have produced a need to apply insecticides more economically and efficiently (Halmer, 1988). In controlling the cabbage root fly (*Delia radicum*) a significant reduction in the necessary quantity of insecticide was achieved by applying the insecticides as a film-coating to cabbage seed (Ester & De Moel, 1992). This paper reports on controlling the onion fly (*Delia antiqua* (Meigen)) in leek with a reduced amount of insecticides. Onion seeds filmcoated with the insecticide benfuracarb have been on the market in The Netherlands for some years. Narkiewicz-Jodko (1991) reported successful control of onion fly in onion by applying carbosulfan or isofenphos as a seedcoating. In a leek crop the onion fly is mainly a problem in seed beds where plants are raised at high densities. Generally transplanting to the production field takes place approximately 12 weeks after sowing. The onion fly attacks the seedling by hollowing out the basal part of the plant resulting in its collapse. Due to the high plant density in the seed bed, neighbouring plants are easily attacked too, resulting in patches of collapsed plants. In the production field plant spacing is much wider and the onion fly is not a real problem. Therefore the effects of a seed film-coating were investigated after sowing in seed beds, according to common practise. A preliminary report has been published (Ester, *et al* 1992).

MATERIALS AND METHODS

All experiments were carried out with the winter leek variety Porino. Film-coating of the seed was done by SUET (Saat- und Erntetechnik, Eschwege, Germany) using a fluidised bed film-coating technique. The film-coating contained polymers to give a dust free product. In order to obtain the same amount of insecticide per seed, rates are expressed per unit of seed, with one unit equalling 250,000 seeds. All treatments contained the same amount of fungicide, namely

thiram at 1 g AI per unit of seed, except the isofenphos/thiram treatment. For this treatment a combined formulation of isofenphos and thiram has been used (powder for dry seed treatment DS 40/10%, containing 40% isofenphos and 10% thiram). The thiram rates were 0.9, 1.4 and 3.6 g AI/unit of seed for the 7, 11 and 14 g AI rates of isofenphos respectively. Untreated seeds were treated as a film-coating with thiram only. Four insecticides were applied at different rates. Benfuracarb (wetttable powder WP 40%) was applied at 20, 27 or 40 g AI per unit seed, carbofuran (suspension concentrate 500 SC) at 27 g AI per unit of seed, imidacloprid (water dispersible powder for slurry treatment, 70% WS) at 14 or 28 g AI per unit of seed and isofenphos at 7, 11 or 14 g AI per unit of seed.

All germination tests in the laboratory were carried out in silver sand (sand test) or in a peatbased potting compost (soil test), using 3 replications of 100 seeds. Trays were placed in germination cabinets at 15°C night and 20°C day. The percentage of normal plants emerging was assessed after 16 days (sand test) or 15 days (soil test).

Insecticide efficacy and/or emergence experiments were carried out at four field sites in The Netherlands with a history of onion fly attack: Rijsbergen (1991; 1992), Berkel-Enschot (1992), Tollebeek (1992) and Haelen (1992). The control treatment in the seed bed consisted of a soil treatment of chlorfenvinphos at 6 l AI per ha and two spray applications with carbofuran at 4.4 l AI per ha. The soil treatment was sprayed and incorporated just before sowing, followed by crop sprayings six and twelve weeks later. Each replicate included one plot without insecticide treatment i.e. film-coated seeds with thiram only. All the experiments were sown with a "Nibex" hand-sowing machine and carried out as a randomized block design.

At Rijsbergen site, where the soil was sandy, the treatments were randomized within three replicates (1991; 1992). Each plot consisted of 12 rows (11 cm between rows) of 4.5 m length in 1991 and 13 rows of 2.50 m length in 1992. The seeds were sown in mid-April in both years.

At Berkel-Enschot site, also a sandy soil, the treatments were randomized within four replicates. Each plot consisted of 14 rows (15 cm between rows) of 3.25 m length. The seeds were sown in mid-April 1992.

At Tollebeek site, a marine loam soil, the treatments were randomized within three replicates. Each plot consisted of 12 rows (14 cm between rows) of 3.75 length. The seeds were sown at the end of April 1992.

At Haelen site, another sandy soil, trials were especially designed to assess field emergence of the insecticide-coated seeds compared to seeds without any insecticide. For each treatment 200 seeds were hand-sown in one row of 3 m length. A fully randomized block design was used with 4 replicates. The seeds were sown at the end of April 1992. The number of emerged plants in each row was assessed four weeks after sowing.

Field emergence on the seedling-bed was assessed by counting six rows of one meter per replicate at Rijsbergen in 1991. In 1992 field emergence was not assessed except at Haelen, due to a defect with the sowing machine.

The damage to all the crops by the onion fly was assessed regularly between six and eleven weeks after sowing by observing the percentage of collapsed plants.

The statistical analysis was performed with the statistical package Genstat.

RESULTS

Germination

Film-coated seeds without insecticide showed no reduced germination or field emergence compared to non-filmcoated seeds (data not shown). Seeds film-coated with benfuracarb and carbofuran in both 1991 and 1992 did not differ in percentage normal plants in laboratory germination tests carried out in 1992 as compared to the control film-coated seeds. Imidacloprid at 28 g AI and isofenphos at 11 g AI and 14 g AI/unit of seed significantly lowered the percentage of normal plants in the sand test in comparison with untreated film-coated seeds. In the soil test only isofenphos-treated seeds showed a significantly lower percentage of normal plants (Table 1.).

TABLE 1. Laboratory germination of filmcoated winter leek seeds in 1992. Percentage normal plants after 16 days in the sand test and after 15 days in the soil test.

Insecticides	Rate g AI/unit	Sand test		Soil test
		1992**	1992	1992
untreated	-	92	93	91
benfuracarb	20	93	92	92
	30	-	92	90
	40	-	91	-
carbofuran	27	-	91	-
imidacloprid	28	78	87	90
isofenphos	7	89	89	85
	11	-	87	-
	14	85	-	-
LSD (p=0.05)		4.8	4.8	4.6

** seed treated in 1991, tested one year later.

In 1991 only benfuracarb at 40 g AI/unit of seed showed significantly lower field emergence compared to the control film-coated seeds. In 1992 only the field emergence of benfuracarb at 20 g AI/unit of seed and imidacloprid-treated seed was not significantly different from the untreated seed (Table 2). Because of a defect in the sowing machine a reliable assessment of the field emergence could be made only at the Haelen site.

TABLE 2. Field emergence of winter leek seeds. Number of seedlings per meter row length four weeks after sowing in Rijsbergen (1991) and Haelen (1992).

Insecticides	Rate g AI/unit	Number of plants	
		1991	1992
film-coating			
untreated	-	32	82
benfuracarb	20	30	78
	30	-	73
	40	27	-
imidacloprid	14	33	-
	28	32	80
isofenphos	7	34	68
	14	29	-
soil + crop treatment			
chlorfenvinphos + carbofuran	6+4.4*	33	-
LSD (p=0.05)		3.2	4.7

* g AI per hectare.

Efficacy

Both the soil plus crop treatment and the seed film-coatings reduced the onion fly damage significantly compared to the untreated control, except at the Rijsbergen site in 1992 (Table 3). In this trial carbofuran showed no difference from the untreated control after 10 weeks. Although the percentages of damaged plants were lower for all other seed coatings, these results were not significant. Control of the onion fly by all seed coatings was not significantly different from the standard soil plus crop treatment, except for the carbofuran coating at the Rijsbergen site in 1992.

TABLE 3. Efficacy of insecticides applied as a seed coating for onion fly control in leek. Percentages of damaged plants 14 weeks (1991) and 10 weeks (1992) after sowing.

Insecticides	Rate g AI/unit	Rijsbergen		Berkel Enschoot	Tollebeek	
		1991	1992	1992	1992 ^{***}	1992
film-coating						
untreated		1.6	7.5	13.5	13.6	13.6
benfuracarb	20	0.03	-	-	0.1	-
	30	-	0.9	0.0	-	0.0
	40	0.03	-	-	-	-
carbofuran	27	-	10.6	1.3	-	0.8
imidacloprid	14	0.10	-	-	-	-
	28	0.03	0.6	0.9	-	0.1
isofenphos	7	0.03	0.3	0.1	0.0	0.0
	11	-	0.4	0.1	-	0.2
	14	0.03	-	-	-	-
soil + crop-treatment						
chlorfenvinphos + carbofuran **	6+4.4	0.03	0.4	0.0	0.0	0.5
LSD ($\alpha = 0.05$)		0.70	7.39	3.76	5.26	5.26

** Control treatment g AI per ha.

*** Seed treated 1991 and sown 1992.

DISCUSSION AND CONCLUSION

The aim of insect control is to avoid economic crop losses. This research demonstrates that, for the control of onion fly in leek, this can be achieved by seed coatings with insecticides. Of the compounds tested, carbofuran failed to give sufficient protection in one trial, possibly due to a longer onion fly flight. Benfuracarb, isofenphos and imidacloprid reduced the amount of onion fly attack to levels comparable to those achieved by the current standard treatment in The Netherlands, a soil treatment with chlorfenvinphos and two crop treatments with carbofuran.

In the laboratory tests phytotoxicity has been observed for the isofenphos seedcoatings at high rates and also at the lowest rate of 7 g AI/unit of seed in the field trials in 1992. As imidacloprid also showed a reduced laboratory germination, only benfuracarb at the lowest rate of 20 g AI/unit seed always gave adequate control without phytotoxicity. Film-coating leek seeds with benfuracarb at the rate of 20 g AI/unit of seed illustrates clearly the possibility to reduce the amount of insecticides required to control the onion fly in a leek crop. Compared to the conventional application, of soil treatment plus two spray applications, a seed-application will reduce the necessary amount of insecticide by 98% to 3 g per 100 m².

In The Netherlands, currently benfuracarb at the rate of 20 g Al/unit of seed has received a clearance to be used as a seed coating of leek. This method of control is recommended as part of the integrated pest management scheme for leek.

REFERENCES

- Ester, A.; Embrechts, A.; Vogel, R. de; Schouten, K. (1992) Tegen zaadcoating kan uievlieg niet op. *Groenten en Fruit/Vollegroondsgroenten*, 44, 10-11.
- Ester, A.; Moel, C.P. de (1992) Controlling cabbage root fly in Brussels sprouts by filmcoating seeds with insecticides. *Proceedings Experimental & Applied Entomology, NEV Amsterdam*, 3, 181-190.
- Halmer, P. (1988) Technical and commercial aspects of seed pelleting and film-coating. In: *Applications to Seeds and Soil*, T.J. Martin (Ed.), *BCPC Monograph No. 39*. Thornton Heath: BCPC Publications, pp. 191-204.
- Narkiewicz-Jodko, J. (1991) Effect of Marshal 25 ST Carbosulfan in control of onion fly *Hylemya antiqua* Meig. and Carrot fly *Psila rosae* F. *Mededelingen van de Faculteit Landbouwwetenschappen van Rijksuniversiteit Gent*, 56/3b 1143-1150.

ERADICATION OF *FUSARIUM* FROM OIL PALM BY SEED TREATMENTS.

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ABSTRACT

Fusarium oxysporum f.sp. *elaeidis* can be present naturally on or within oil palm seeds and a low proportion of infested seed gives rise to infected plants. Vacuum infiltration and soaking for 7 days with captafol or a formulation of prochloraz plus carbendazim effectively eradicated the pathogen. Seed treatment should be used as a precaution to reduce the possibility of the disease being introduced into previously disease free regions.

INTRODUCTION

Vascular wilt, caused by *Fusarium oxysporum* f.sp. *elaeidis* (F.o.e.) is the most serious disease of oil palm in West Africa (Turner, 1981). The disease appears to be absent from major producing areas in S.E. Asia such as Malaysia and Indonesia but has occurred in Brazil (Van de Lande, 1984) and Ecuador in 1986 (Renard & de Franqueville, 1989). The origin of these outbreaks is not known but seed-borne transmission is likely; the pathogen can be present along with *Fusarium solani* on the seed coat (Locke & Colhoun, 1974) or on the kernel surface inside seeds (Flood et al., 1990). Also, isolates of the pathogen from Ivory Coast, Brazil and Ecuador are vegetatively compatible, according to results with nitrate non-utilising (*nit*) mutants which may indicate a common origin; seeds from Ivory Coast were used to plant some of the affected plantations in South America (Flood et al., 1992). The pathogen can survive normal routine seed processing (soaking for 7 days at 25°C, heating for 75 days at 39°C followed by further soaking at 25°C for 7 days) and contaminated seed can give rise to infected plants albeit at a low frequency. Consequently, an effective fungicidal seed treatment was required which could be incorporated with normal seed processing.

EXPERIMENTAL

Natural contamination of oil palm seed by F.o.e. can vary considerably between seed consignments and between individual seeds (Flood et al., 1990) and thus, seeds from susceptible crosses were artificially inoculated (Flood et al., 1994) to allow quantification and statistical analysis.

Quantification of *Fusarium oxysporum* on oil palm seeds

Following inoculation, levels of *F. oxysporum* on seed coats and kernel surfaces were quantified as mean colony forming units (cfus) per shell or per kernel using dilution and plating techniques (Flood et al., 1990).

Fungicide treatments

A suspension of captafol (Sanspor 50% a.i. ICI Agrochemicals) was prepared (1 g a.i./litre water plus 0.1ml Tween 20) and applied as either a 7 day soak (change of fungicide every day) or as a vacuum infiltration

treatment. In the latter treatment, captafol plus seeds were placed in a vacuum chamber (Edwards-Pearce freeze drier) in which the air pressure was reduced until the liquid began to boil (1000 - 1200 Pa). This process was repeated three times with alternating repressuration. Following vacuum infiltration, the seeds were soaked for 7 days with a daily change of captafol.

Seeds were also vacuum treated with a suspension (1 g a.i./litre water plus 0.1ml Tween 20) of benomyl (Benlate 50% a.i., Dupont) using the method described above and then soaked for 7 days with a daily change of benomyl.

After fungicide treatment, the mean cfus of *Fusarium oxysporum* per seed were determined and compared with those obtained after seed had received a routine 7 day soak with water. Vacuum infiltration plus soaking with captafol eradicated *F. oxysporum* from shells and kernels (Table 1) but soaking alone with this fungicide failed to eradicate the pathogen from kernel surfaces. Benomyl significantly reduced *F.oxysporum* from seed coats and kernels but it failed to eradicate it.

TABLE 1 Levels of *Fusarium oxysporum* on seed coats and kernels following fungicide treatments.

Treatment	Mean cfus/shell	Mean cfus/kernel
Captafol soak (daily change of fungicide for 7 days)	0 ^b	155 ^{ab}
Vacuum infiltra- tion with captafol plus 7 day soak	0 ^b	0 ^b
Vacuum infiltra- tion with benomyl plus 7 day soak	22 ^b	54 ^b
Water soak (daily change of water for 7 days)	2340 ^a	274 ^a

cfus = colony formg units (Flood et al., 1989).
 Values represent a mean of 14 replicates.
 Within each column, values with the same letter are not significantly different at 1% level using STD test for non-parametric data (Sokal & Rohlf, 1981).

Captafol and a formulation of prochloraz and carbendazim (Sportak Alpha, Schering Agrochemicals) were chosen for further experimentation as a post-heat treatment in order that fungicide treatments could be incorporated into routine seed processing protocols. Hence, the fungicides (1g a.i./litre water plus 0.1ml Tween 20) were vacuum infiltrated into

inoculated seeds following heat treatment. The seeds were soaked for 7 days (daily change of fungicide) and air dried. They were cracked aseptical and shells and kernels plated onto *Fusarium* selective medium and incubated for 14 days at 25°C. Following incubation, the presence of *F. oxysporum* on seeds and kernels was determined and compared with seeds which had received heat treatment and water soaking only. Vacuum infiltration with both fungicides eradicated *F.oxysporum* from shells and kernels (Table 2).

TABLE 2 Presence of *F.oxysporum* on heat- treated inoculated seeds following fungicide vacuum infiltration.

Treatment	Shell	Kernel
Control (7 day soak in water)	45 ⁺ a	45 a
Vacuum infil- tration plus 7 day soak with captafol.	0 b	0 b
Vacuum infil- tration and 7 day soak with prochloraz plus carbendazim.	0 b	0 b

+Values represent results from 50 replicate seeds.
Within each column, values followed by the same letter
are not significantly different using X^2 analysis (< 0.01).

Effect of fungicide treatment on seed germination and on plant development

Artificially inoculated seeds which had been heat treated and then treated with either fungicide were incubated in plastic bags at 25°C for approximately 3 weeks. Germination rates of 60 seeds treated or not treated with fungicide were very similar: 70% for fungicide treated seeds as compared to 68% for untreated seeds ($P>0.05$ using x^2 analysis). No abnormalities in root or shoot development were evident following fungicide treatment either when the young seedlings were transplanted into trays or at the 1-2 leaf stage when the seedlings were transferred to individual pots. All plants appeared to grow normally throughout the 9 month period of the experiment.

DISCUSSION

Vascular wilt pathogens such as *Verticillium* and *Fusarium* are generally considered as soil-borne fungi; their dissemination is mostly by water or wind erosion of soil, irrigation or movement of man and equipment. Consequently, their disease spread is ususally confined to a restricted area. However, long distance movement can also occur through the movement

of infected vegetatively propagated material or seed and many of the *formae speciales* of *Fusarium oxysporum* have also been demonstrated to be seed-borne (Gambogi, 1983).

F. oxysporum f. sp. *elaeidis* can be naturally present on the seed coats and kernel surfaces of oil palm seeds (Locke & Colhoun, 1974; Flood et al., 1990) but embryo infection has not yet been demonstrated. Oil palm seeds routinely undergo heat treatment (at 39°C) to induce germination and high temperatures have proved to be effective seed treatments for the removal of several other *formae speciales* e.g. *lycopersici* (Besri, 1978). However, although heat treatment drastically reduced the populations of *F. oxysporum* and *F. solani* on oil palm shells and kernels, neither of these fungi were eradicated (Flood et al., 1994).

Also, *F. oxysporum* was reisolated from stem base tissue of 2 out of 60 plants grown from seeds inoculated with *F. o. e.* and these plants developed characteristic vascular necrosis (Flood et al., 1994). Thus, with a low initial inoculum (mean 7 cfus per kernel and 40 cfus per shell) infected plants were produced, albeit at a low frequency. Although direct comparisons are difficult to make, contamination levels of up to 5×10^3 cfus per seed and up to 100 cfus per kernel have been reported from naturally contaminated seeds (Flood et al., 1990). Thus, the pathogen may be introduced into new areas in this way as is likely to have already occurred in South America.

Consequently, as a precautionary measure, fungicide treatment of seeds is required and eradication of the pathogen on kernel surfaces is essential.

Vacuum infiltration of benomyl effectively reduced *Fusarium oxysporum* on both oil palm seed coats and kernels but it failed to eradicate these fungi. Haware et al. (1978) similarly reported that benomyl alone reduced *F. oxysporum* f. sp. *ciceri* from chickpea seed but it failed to eradicate the pathogen. In contrast, vacuum infiltration with captafol completely eradicated *F. oxysporum* from oil palm seed coats and kernels and had no phytotoxic effects on seed germination or seedling development, but captafol has been withdrawn from recommended use. Vacuum infiltration plus a 7 day soak with a formulation of prochloraz plus carbendazim was shown to be as effective as captafol and could be used for batches of seed exported from Africa to countries which are currently wilt-free such as Malaysia, Indonesia, Papua New Guinea and India.

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REFERENCES

- Besri, M. (1978) Phases de la transmission de *Fusarium oxysporum* f.sp. *lycopersici* et de *Verticillium dahliae* par les semences de quelques variétés de tomate. *Phytopathologische Zeitschrift* **93**, 148-163.
- Flood, J.; Cooper, R.M.; Lees, P.E. (1989) An investigation of pathogenicity of four isolates of *Fusarium oxysporum* from South America, Africa and Malaysia to clonal oil palm. *Journal of Phytopathology*, **124**, 80-88.
- Flood, J.; Mepsted, R.; Cooper, R.M. (1990) Contamination of oil palm pollen and seeds by *Fusarium* spp. *Mycological Research*, **94**, 708-709.
- Flood, J.; Whitehead, D.S.; Cooper, R.M. (1992) Vegetative compatibility and DNA polymorphisms in *Fusarium oxysporum* f.sp. *elaeidis* and their relationship to isolate virulence and origin. *Physiological and Molecular Plant Pathology*, **41**, 201-215.
- Flood, J.; Mepsted, R.; Cooper, R.M. (1994) Population dynamics of *Fusarium* species on oil palm seeds following chemical and heat treatments. *Plant Pathology* (in press).
- Gambogi, P. (1983) Seed transmission of *Fusarium oxysporum*: epidemiology and control. *Seed Science & Technology*, **11**, 815-827.
- Haware, M.P.; Nene, Y.L.; Rajashwari, R. (1978) Eradication of *Fusarium oxysporum* f. sp. *ciceri* transmitted in chickpea seed. *Phytopathology*, **68**, 1364-1367.
- Locke, T.; Colhoun, J. (1974) *Fusarium oxysporum* f. sp. *elaeidis* as a seed-borne pathogen. *Transactions of the British Mycological Society* **60**, 594-595.
- Renard, J.L.; De Franqueville, H. (1989) Oil palm vascular wilt. *Oleagineaux*, **44**, 341-347.
- Sokal, R.R.; Rohlf, F.J. (1981) *Biometry: The Principles and Practice of Statistics in Biological Research*. (2nd Edition) New York: Freeman and Company.
- Turner, P.D. (1981) *Oil Palm Diseases and Disorders*. Oxford: Oxford University Press.
- Van de Lande, H. (1984) Vascular wilt disease of oil palm (*Elaeis guineensis* Jacq.) in Para Brazil. *Oil Palm News*, **28**, 6-10.

FIELD EMERGENCE OF PEAS AS AFFECTED BY SEED QUALITY AND FUNGICIDE SEED TREATMENTS.

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ABSTRACT

Laboratory germination and field emergence of pea (*Pisum sativum L.*) was studied in three seed lots using fungicide film-coating. The best field emergence was obtained with the lot which showed the best results in the laboratory germination tests, and which also had the lowest electroconductivity. The level of field emergence equalled the laboratory germination of the untreated seed if the seeds were film-coated with the fungicide combinations of oxadixyl+cymoxanil+carbendazim or metalaxyl+thiabendazol+thiram. The use of oxin-copper had no significant effect on field emergence if added to the mixtures. Thiram or thiram+oxin-copper did not lead to good field stands when used on the weaker lots.

INTRODUCTION

Field emergence of pea is important in relation to field establishment and final yield (Trawally *et al.*, 1984). Emergence cannot be predicted from the standard germination test (Duczmal & Minicka, 1989). Field emergence is determined by seed quality, seed-borne pathogens and soil conditions (biotic and abiotic). The complex interactions between these factors make it impossible to study them separately. Fungicide seed treatments may contribute to a good field stand by protecting the germinating seed from seed borne and soil borne pathogens.

Several different seed treatments are commercially available for peas (F. Haquin, 1987). In this study we tested the effect of fungicides applied in a film-coating on laboratory germination and on field emergence. Root rot development was not assessed as several reports indicate that chemical control does not effectively control soil borne root rot (Kraft, 1982; van Loon & Oyarzun, 1988).

MATERIALS AND METHODS

Seed treatment

The three pea lots used in this study were produced in central France in 1992, and were selected for their differences in the levels of germination and the levels of seed borne organisms. There were three cultivars, Florado (lot A), Spartan (lot B) and Ninado (lot C); all these varieties had wrinkled seeds. Thousand seed weight was 174 g for lot A, 158 g for lot B and 105 g for lot C. Seeds were tested for levels of seed borne infection by "Ministere de l'Agriculture et de la forêt service de la protection des vegetaux" Angers (France) in september 1992. Seeds were film-coated with 6 fungicide combinations (table 1) in batches of 400 g using a laboratory fluidised bed coater. Polymers were used to give a uniform and dust free product.

The composition of the fungicide formulations used was; Pulsan TS Pepite (40% oxadixyl, 16% cymoxanil), Bavistine (50% carbendazim), Apron combi 453 (233 g/l metalaxyl, 120 g/l thiabendazol, 100 g/l thiram), Aatiram 75 (75% thiram), Quinolate Pro FL (120 g/l carbendazim, 120 g/l oxin-copper), Quinolate 400 (400 g/l oxin-copper). Oxin-copper was used as a split factor in the trial.

TABLE 1. Fungicide seed treatments of pea seeds in gram AI per kg of seed.

Treatment	1	2	3	4	5	6
AI						
oxadixyl	0.60	-	-	0.60	-	-
cymoxanil	0.24	-	-	0.24	-	-
carbendazim	1.00	-	-	0.30	-	0.30
metalaxyl	-	0.80	-	-	0.80	-
thiabendazol	-	0.41	-	-	0.41	-
thiram	-	0.34	0.30	-	0.34	0.30
oxin-copper	-	-	-	0.30	0.30	0.30

Germination tests

Seeds were germinated in 4 replications of 50 seeds according to the ISTA method (sand/perlite at 20°C) and in a cold test (sand/perlite for 10 days at 8°C followed by 20°C). Seeds were sown in the field by hand in a completely randomised design in 7 replications of 100 seeds on April 1, 1993. Observations on emergence were done on 20 days after sowing (total plants), 26 days after sowing (total plants) and 50 days after sowing (normal plants). The soil was a sandy clay in Enkhuizen. Mean day temperatures were below 10 °C during the first three weeks after sowing.

Conductivity was determined in a bulk conductivity test with 2 replicates of 50 seeds at 20 °C for the untreated seeds.

RESULTS

Levels of seed infection are given in table 2. Laboratory germination under standard conditions (table 3) was not affected by the fungicide treatments for lot B and lot C. For lot A, treatments 1 and 4 showed lower germination than the control; treatments 5 and 6 germinated better. In the cold test all fungicide treatments had a negative effect on germination for lot B; germination of lot C was not affected. The effects for lot A were variable; compared to the control germination was lower in treatment 4, and better in treatments 2,3 and 5.

TABLE 2. Percentage fungus infected seeds in the seed lots used in the trials.

fungus	lot A	lot B	lot C
<i>Botrytis cinerea</i>	9	6	10
<i>Penicillium spp.</i>	66	7	0
<i>Alternaria spp.</i>	2	27	23
<i>Stemphyllium spp.</i>	1	3	5
<i>Mycosphaerella pinodes</i>	1	15	0.5

Other pathogens detected in the test (*Fusarium spp.*, *Trichoderma spp.*, *Phoma medicaginis*, *Cladosporium spp.*) were at levels of 1% or below for the 3 lots.

TABLE 3. Percentage germination in sand/perlite at 20°C and in a cold test (10 days at 8°C followed by 20°C), and field emergence of pea seed lots A, B and C with the different seed treatments.

Treatment (see TABLE 1)	Germination at 20 °C		Cold test	Field emergence	
	7 days normal	10 days normal	15 days normal	20 days total	50 days normal
Lot A					
1	54	70	49	72	85
2	54	75	65	63	80
3	56	78	65	60	64
4	50	71	43	63	81
5	81	87	71	63	81
6	73	90	80	40	44
control	63	80	51	28	28
Lot B					
1	82	88	69	66	82
2	75	83	70	63	81
3	68	82	73	63	72
4	74	82	71	62	82
5	71	85	73	63	78
6	76	84	71	59	64
control	71	79	79	43	40
Lot C					
1	99	99	93	84	96
2	98	98	91	82	93
3	99	99	94	85	95
4	95	96	87	83	97
5	96	97	90	84	93
6	97	97	92	80	91
control	98	98	90	74	72
LSD at p=0.05	6.5	6.3	5.1	8.1	6.1

Field emergence was improved by all fungicide treatments. Compared to the controls, field emergence was increased from 28% to 85% for lot A, from 40% to 82% for lot B and from 72% to 97% for lot C. For lot A and lot B the best emergence was obtained with treatments 1, 2, 4 and 5. For lot C all treatments gave the same level of field emergence. The correlation between laboratory germination and field emergence was only 0.477.

Conductivity, as determined for the untreated seeds, was 34.81 $\mu\text{s}/\text{cm}$ (lot A), 32.58 $\mu\text{s}/\text{cm}$ (lot B) and 17.82 $\mu\text{s}/\text{cm}$ (lot C).

DISCUSSION

No correlation existed between standard laboratory germination or the cold test and field emergence. For lot A the highest level of germination in the laboratory test (treatment 6) resulted in the lowest field emergence. A very high correlation was obtained between conductivity and field emergence for the untreated seed ($r^2 = 0.978$). For all lots, treatments 1, 2, 4 and 5 gave good field stands. For the best lot (lot C), all fungicide treatments gave the same level of field emergence. It is striking that the germination level of the untreated seed in the laboratory (10 days, 20 °C) was always matched by the field emergence if the seed was correctly treated with fungicides.

The high incidence of *Penicillium* on lot A could be due to unfavorable post harvest weather conditions which lead to low vigour.

Even with weak lots good field stands were obtained if seeds were treated with the oxadixyl/cymoxanil/carbendazim or metalaxyl/thiabendazol/thiram mixtures. Addition of oxin-copper to these mixtures did not improve stands any further. To obtain seeds correctly treated with these mixtures, film-coating techniques are needed for their application.

REFERENCES

- Duczmal, K.W.; Minicka, L. (1989) Further studies on pea seed quality and seedling emergence in the field. *Acta horticulturae*, **253**, 239-246.
- Haquin, F. (1987) Traitements de semences: ça bouge aussi pour les oléoprotéagineux. *Semences et Progrès*, **53**, 3-12.
- Kraft, J.M. (1982) Field and Greenhouse Studies on Pea Seed Treatment. *Plant disease*, **66**, 798-800.
- van Loon, J.J.A.; Oyarzun, P. (1988) Zaadinfectie bij droge erwten, een potentiële bron van verspreiding van voetziekten in de erwtenenteelt. *Gewasbescherming*, **19** (2), 51-60.
- Trawally, B.B.; Noonan, M.J.; Close, R.C. (1984) The effect of seed treatment on plant establishment, downy mildew and yield of peas. *Proceedings of the 37th New Zealand weed and pest control conference*, 163-166.

EFFECT OF THE PERIOD BETWEEN SOWING AND TRANSPLANTING ON CABBAGE ROOT FLY (*DELIA RADICUM*) CONTROL IN BRASSICAS WITH CHLORPYRIFOS FILM-COATED SEEDS

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ABSTRACT

Cabbage root fly (*Delia radicum*) control with chlorpyrifos film-coated seed has been successfully practised in the Netherlands for the last 2 years. The influence of the period between sowing and transplanting (27 to 133 days) on the resulting control of cabbage root fly was assessed for cauliflower and Brussels sprouts at two sites in the Netherlands. Infection rate was extremely high at both sites. The percentage of dead plants was the lowest for the chlorpyrifos film-coated seed in all cases. The root damage index (RDI%) was lower for chlorpyrifos film-coated seed than for the control in all cases. Control by chlorpyrifos film-coating is effective for a period of up to 133 days between sowing and transplanting. Longer periods were not tested.

INTRODUCTION

Cabbage root fly (*Delia radicum*) is an important pest in horticultural brassicas. Film-coating seeds with chlorpyrifos gives reliable control of cabbage root fly and reduces the amount of insecticide needed (Kosters *et al* 1993; Ester *et al* 1993). Growing practice for brassicas, and more specifically for cauliflower and Brussels sprouts, varies considerably in the Netherlands. The time between sowing and transplanting can vary from 4 to 18 weeks (de Moel, 1993). Cabbage root fly control by chlorpyrifos also depends on soil and weather conditions if applied onto the soil at the moment of transplanting (Rouchaud *et al*, 1989). With the much reduced quantity of chlorpyrifos used in a film-coating compared to field treatment this dependency may be greater.

In this study we investigated the effect of a period of 27 to 133 days between sowing and transplanting of plants in the field on control of cabbage root fly by chlorpyrifos film-coated seed. Two fields with different soil types and expected high levels of cabbage root fly attack were used.

MATERIALS AND METHODS

Treatments

Seeds of the cauliflower variety Lindurian and Brussels sprouts variety Oliver were film-coated with fungicides only or with fungicides and insecticide. The standard commercial treatments as registered in the Netherlands were used (Table 1). Three treatments were compared on all sowing dates; fungicide control, chlorpyrifos film-coating and granulate treatment with 1 g of 5% chlorpyrifos granules per plant (0.05g a.i./plant) applied at transplanting to plants raised from fungicide-treated control seed.

TABLE 1. Film-coating treatments used in the experiments.

Treatment	AI	treatment rate (AI)
fungicide control	thiram	2 g/kg seed
	carbendazim	1 g/kg seed
	iprodione	5 g/kg seed
chlorpyrifos film-coating	thiram	2 g/kg seed
	carbendazim	1 g/kg seed
	iprodione	5 g/kg seed
	chlorpyrifos	0.096 g/1000 seeds

Field experiments

Plant raising

Plants were sown on six different occasions, viz December 3, January 5, January 29, February 19, March 11 and March 26 which gave differences in the time between sowing and transplanting in the field from 27 to 133 days (Table 2). Plants at all sowing dates were sown and raised in modules with a peat-based potting compost by a commercial plant raiser in the Netherlands, according to standard Dutch horticultural practice.

TABLE 2. Sowing dates and transplanting dates for the cauliflower and Brussels sprouts.

Field site	Sowing dates	Transplanting date	Period between sowing and transplanting (days)
Westmaas	cauliflower: 3/12; 5/1; 29/1; 19/2.	April 15	133, 100, 76, 55
	sprouts: --; 5/1; 29/1; 19/2.		
Prinsenbeek	cauliflower and sprouts: 5/1; 29/1; 19/2; 11/3; 26/3.	April 22	107, 83, 62, 42, 27

Field trials

Field sites were situated at Westmaas, which had a light clay soil, and Prinsenbeek, which had a sandy soil. Westmaas was selected for its known history of early cabbage root fly infection, Prinsenbeek for its light and drought-sensitive soil. Cauliflower and Brussels sprouts were transplanted separately by hand in randomised complete blocks combined over sowing dates. The trial had 5 replications of 65 plants at Westmaas and 4 replications of 72 plants at Prinsenbeek. Number of dead plants was assessed by eye at Westmaas 36, 41 and 48 days after transplanting (DAP), and at Prinsenbeek 21, 29, 35 and 41 DAP.

Mean root damage index (RDI%) was assessed 43 DAP in Westmaas and 49 DAP in Prinsenbeek. Ten complete root systems per replication were harvested and, after washing the surface area of root cortex, damage was assessed by eye. Each root system was then ascribed to one of six categories (0% damage, 1-10% damage, 20-30% damage, 40-60% damage, 70-80% damage, 90-100% damage). The mean root damage index was calculated by multiplying the number of plants in each category by the mean value of that category, adding all of these results together and then dividing by the total number of plants assessed per plot

(Lole, 1992). As an example, a 30% damage level in cauliflower results in considerable crop loss (Long, 1992).

RESULTS

Cabbage root fly attack at 43 DAP for Westmaas and 49 DAP for Prinsenbeek, and the RDI% are presented in figures 1 to 4. The LSD is presented for $p=0.05$.

Westmaas

Cauliflower

Film-coating with chlorpyrifos, and the granulate treatment reduced the percentage of dead plants 48 DAP and the RDI% compared to the controls. The RDI% for the last sowing date was lower for the granulate treatment than for the film-coating treatment (Fig. 1).

Brussels sprouts

Percentage dead plants in the controls was much lower for Brussels sprouts than for cauliflower. The RDI% was reduced to the same levels by the chlorpyrifos film-coating and by the granulate treatment, except on the last sowing date when plants from the granulate treatment were more extensively damaged (Fig.2).

Prinsenbeek

Cauliflower

The percentage dead plants 49 DAP was very high in the controls on all sowing dates. This percentage was reduced to almost zero by the chlorpyrifos film-coating treatment on all five sowing dates, which was lower than the percentage with granulate treatments. No difference in the RDI% was found between the film-coating and the granulate treatments for the first three sowing dates, for the last two sowing dates the RDI% for the granulate treatment was lower (Fig.3).

Brussels sprouts

Both percentage dead plants and RDI% were lower for the chlorpyrifos film-coating and the granulate treatment than in the control on all sowing dates. For some sowing dates control by the chlorpyrifos film-coating was better than with the granulate treatment (Fig.4).

DISCUSSION

Cabbage root fly attack was extremely high in the Netherlands in 1993 (Anon, 1993). This allowed an excellent but severe test of the cabbage root fly control methods. The number of plants killed by cabbage root fly in the plots with chlorpyrifos film-coated seed was very low and was unaffected by sowing date. The RDI% of cauliflower was higher in Westmaas when the sowing date was close to the transplanting date. This may be due to plants from these last dates being smaller and thus more vulnerable to attack. This also is illustrated by the higher RDI% for the controls of both cauliflower and Brussels sprouts on the last sowing date. In Prinsenbeek the RDI% for cauliflower exceeds 30 for both chlorpyrifos film-coating and granulate treatments on all sowing dates.

Surprisingly the RDI% for granular treatments tended to be higher than for film-coating. Dead plants which were selected for RDI assessment were rated as 100% damaged which led to a higher RDI%. The higher RDI% for the granular treatments may be because egg laying by cabbage root flies had already started before the transplanting date. Plants from film-coating were protected from the moment of sowing while the effect of granular treatment only starts after the insecticide has leached from the granules. Granulate application often leads to slight phytotoxic effects on the plants which delays establishment. This short period may be critical for good control. To understand the different control mechanisms from film-coating or granulate application combined field work and analysis of chemical residues in the plant is needed. Analytical work in 1993 at Zaadunie indicated that chlorpyrifos was present in the plant roots from film-coated seed at the moment of transplanting for all sowing dates (personal communication).

In this work it was proven that cabbage root fly control with a film-coating at 0.96 g chlorpyrifos per 1000 seeds gave comparable or better control than the standard granular treatment with 50 g chlorpyrifos per 1000 plants. The effect was irrespective of the period between sowing and transplanting. It was also shown that this year the level of control obtained in cauliflower at one site was not sufficient to reduce the RDI% to a low enough level to avoid economic losses. In years with heavy infestation a second treatment in the first period after transplanting may be necessary if granulate or film-coating is used. However, it was reported that in practice in the Netherlands in 1993 better control was obtained with the film-coating treatments than with the granulate treatments (Anon, 1993, Vader, 1993).

REFERENCES

- Anon. (1993) Zaadcoating by spuitkool geeft afdoende bescherming. *Groenten + Fruit*, no. 25, p. 13.
- Ester, A; Hofstede, S.B.; Kusters, P.S.R.; de Moel, C.P. (1993) Film-coating of cauliflower seed (*Brassica oleracea L. var botrytis L.*) with various insecticides to control the cabbage root fly (*Delia radicum*). *Crop Protection* (in press).
- Kusters, P.S.R.; Hofstede, S.B.; Ester, A. (1993) Effects of film-coating Brussels sprouts seeds with various insecticides on germination and on the control of cabbage root fly (*Delia radicum*). In: *Fourth international workshop on seeds: basic and applied aspects of seed biology*, Vol.3., Daniel Come & Francoise Corbineau (Eds.), Université Pierre et Marie Curie, Paris, 1081-1086.
- Lole, M.J. (1992) Control of cabbage root fly on brassicas with 'Gigant' seed dressing. ADAS Contract report C001/158.
- Long, E. (1992) Dressing beats cabbage root fly. *Grower*, 188, (8), 24- 25.
- Moel de, C.P. (1993) Teelt van bloemkool. PAGV teelthandleiding no. 51. Proefstation voor de Akkerbouw en de Groenteteelt in de Vollegrond, Lelystad, the Netherlands.
- Rouchaud, J; Gustin, F; van de Steene, F.; Pelerents, C; De Proft, M.; Seutin, E.; Vanparys, L.; Benoit, F.; Ceustermans, N. (1989) Soil metabolism of insecticides and their protection efficiency. *Revue de l'Agriculture*, 42, 1309-1326.
- Vader, R. (1993) Resultaat legt sceptici het zwijgen op. *Groenten + Fruit*, no. 31, p. 9.

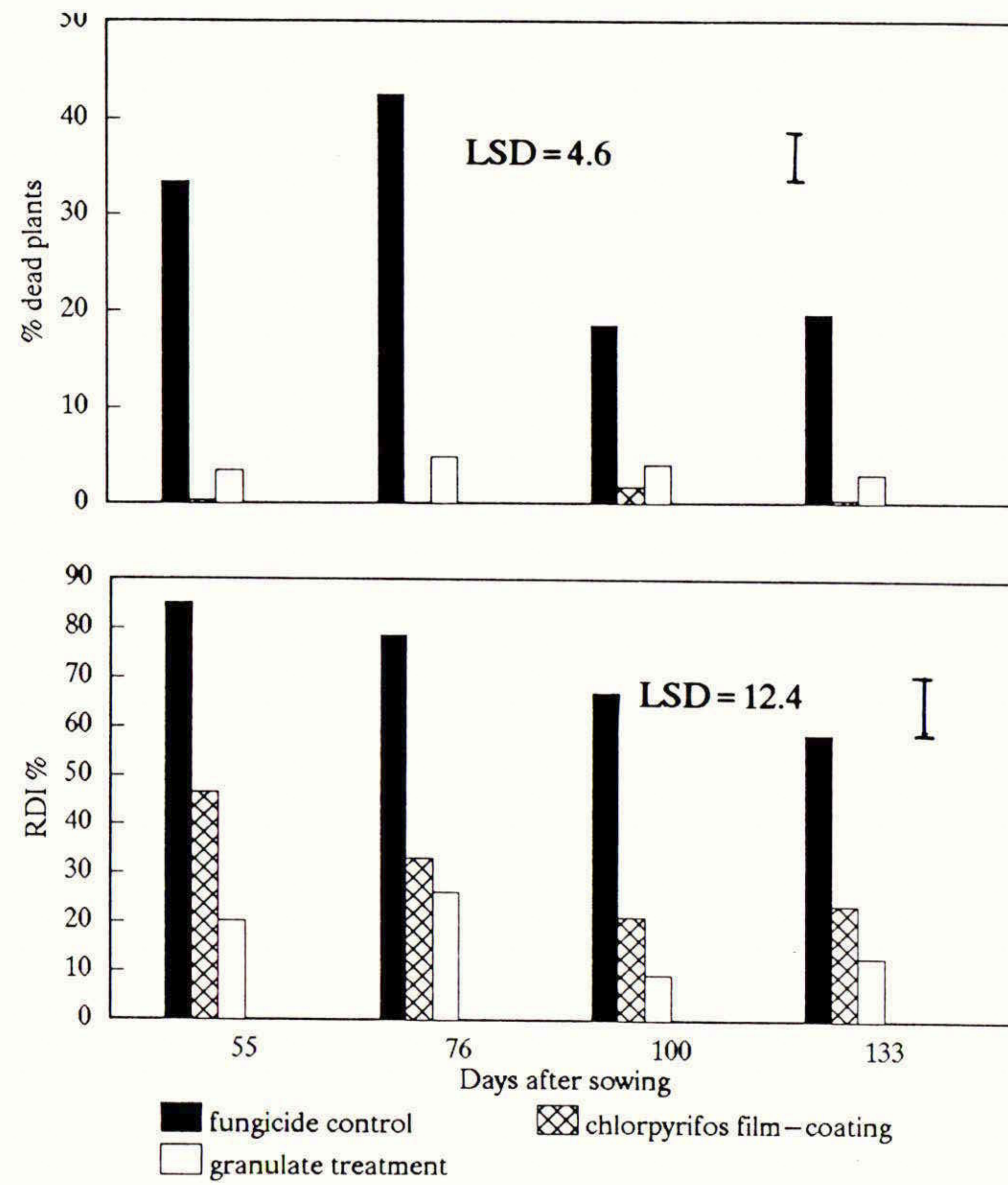


Figure 1. Percentage dead plants from cabbage root fly 48 DAP and RDI% 43 DAP for cauliflower at Westmaas. Transplanting date April 15, 4 sowing dates.

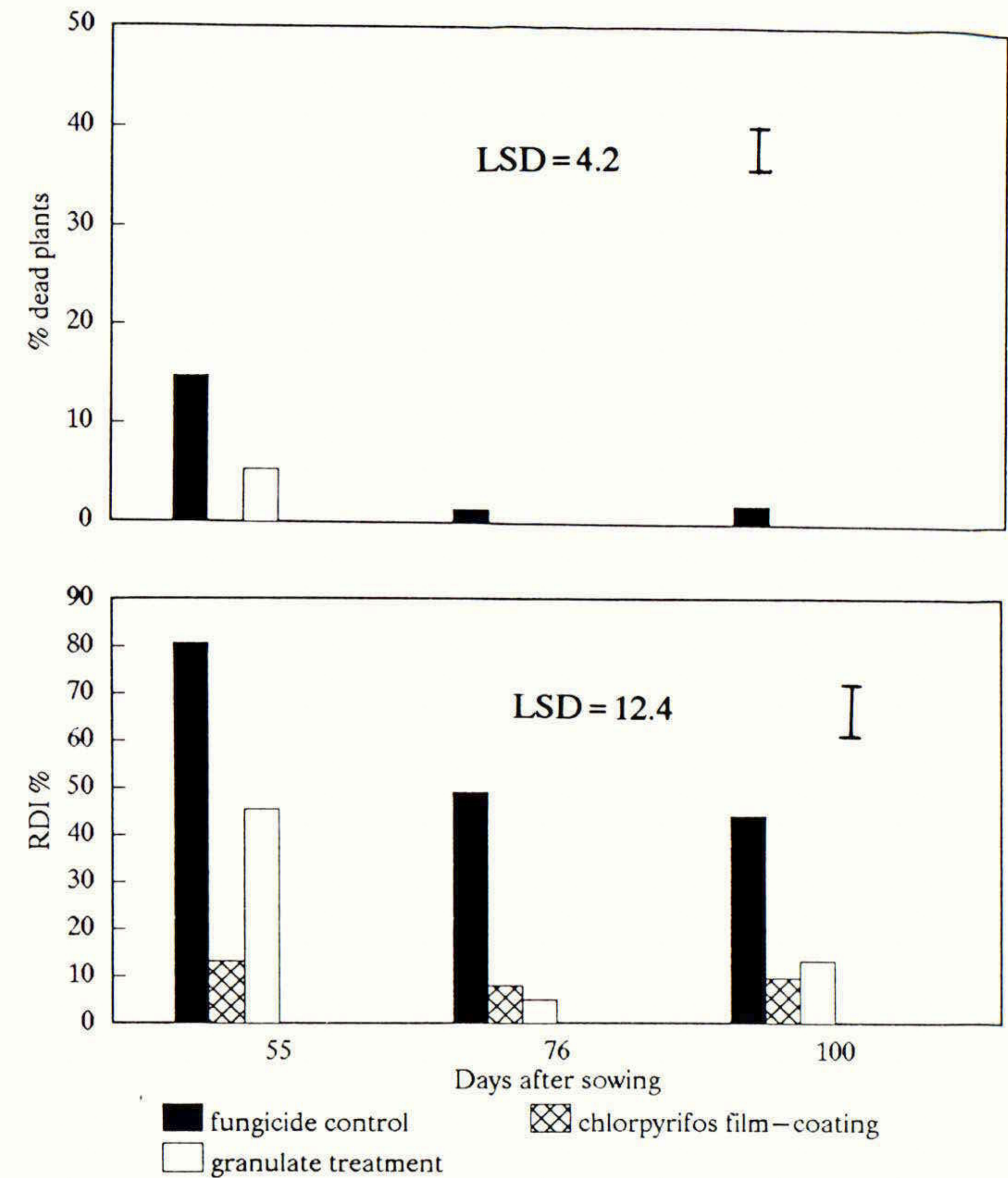


Figure 2. Percentage dead plants from cabbage root fly 48 DAP and RDI% 43 DAP for Brussels sprouts at Westmaas. Transplanting date April 15, 3 sowing dates.

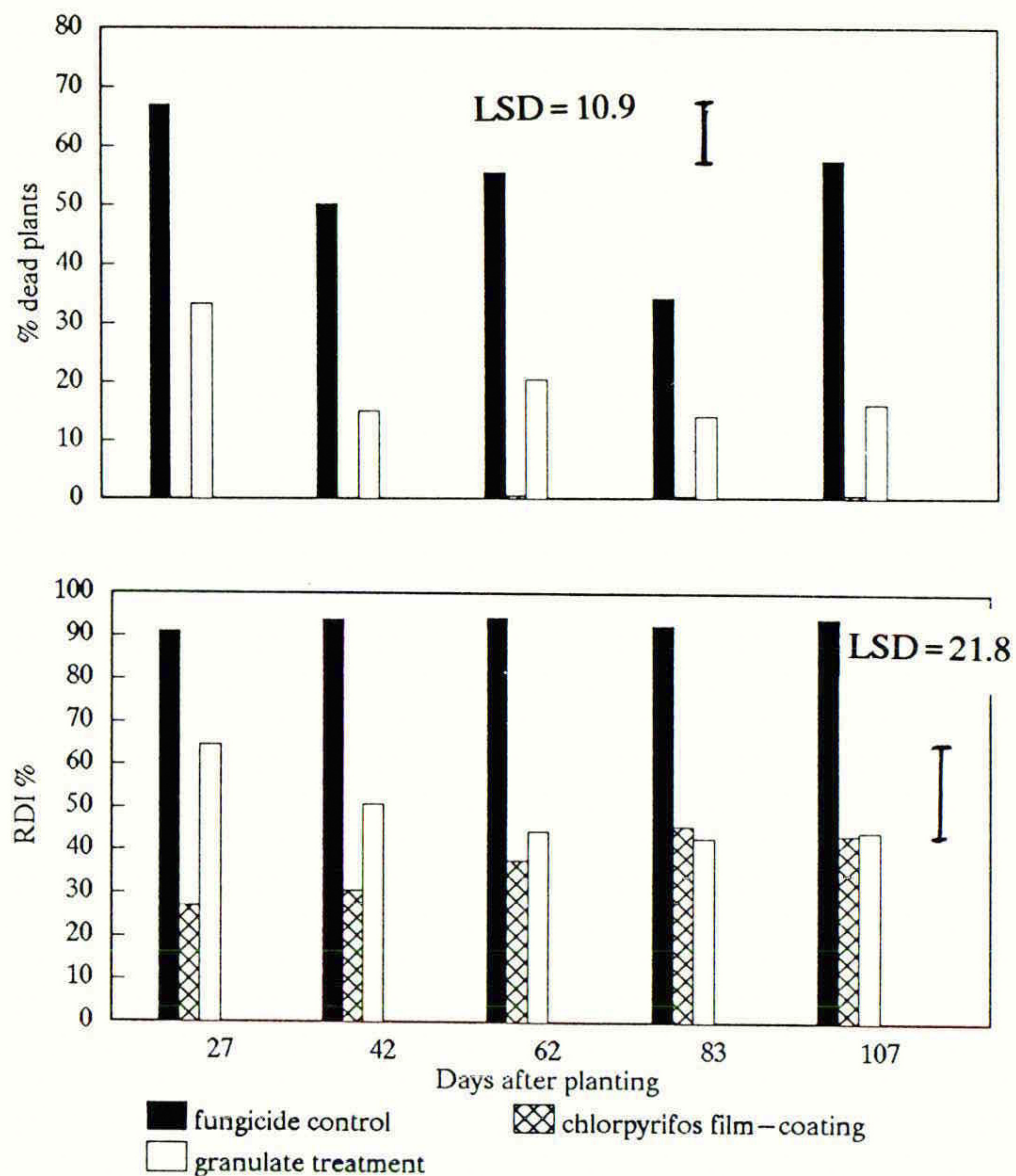


Figure 3. Percentage dead plants from cabbage root fly 35 DAP and RDI% 49 DAP for cauliflower at Prinsenbeek. Transplanting date April 22, 5 sowing dates.

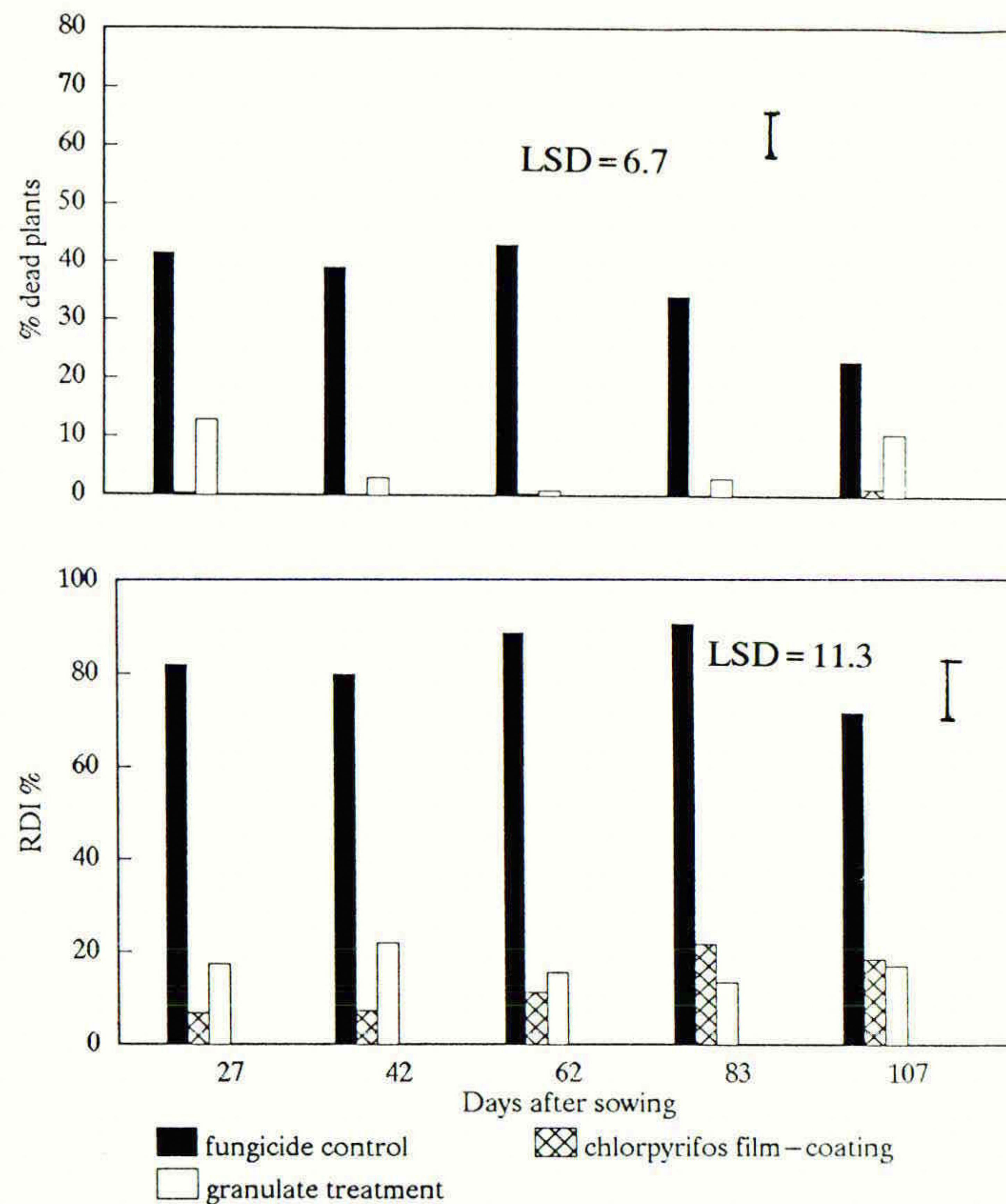


Figure 2. Percentage dead plants from cabbage root fly 35 DAP and RDI% 49 DAP for Brussels sprouts at Prinsenbeek. Transplanting date April 22, 5 sowing dates.