Neth. J. Pl. Path. 77 (1971) 42–54

Laboratory and greenhouse evaluation of a new systemic fungicide, N,N'-bis-(1-formamido-2,2,2-trichloroethyl)-piperazine (CELA W 524)

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Accepted 7 December 1970

Abstract

The new systemic fungicide N,N'-bis-(1-formamido-2,2,2-trichloroethyl)-piperazine (CELA W 524) was shown to display a moderate to distinct fungitoxic activity in vitro towards several pathogenic and non-pathogenic fungi. Depending on the inert ingredients present[2], the available formulations proved to be either rather phytotoxic or virtually non-phytotoxic. Pre-infectional spraying with the non-phytotoxic formulation provided complete protection of barley, bean, cucumber, pea and tomato plants against barley powdery mildew, bean rust, cucumber powdery mildew and cucumber scab, pea powdery mildew and tomato leaf mould, respectively. Some suppression of disease symptoms – although only at high concentrations of CELA W 524 – was observed in the case of leaf spot in pea plants. Upon post-infectional treatment disease control was less pronounced, although powdery mildew diseases and tomato leaf mould were effectively suppressed. When applied via the roots CELA W 524 proved to be systemically active, successfully protecting barley plants against powdery mildew, and cucumber plants against powdery mildew and cucumber scab.

Introduction

In 1967 Dr W. Ost of C. H.Boehringer Sohn, Chemische Fabrik, Ingelheim am Rhein (Germany), synthesized a new systemic fungicide, N,N'-bis-(1-formamido-2,2,2-trichloroethyl)-piperazine (Fig. 1), coded CELA W 524, which was shown to be particularly active against diseases caused by obligate parasites (powdery mildews, rusts) (Schicke and Veen, 1969). Therefore, experiments with CELA W 524 were initiated to test its fungitoxicity in vitro and in vivo, its phytotoxicity as well as its ability to systemically protect plants against fungal infection.

Materials and methods

In fungitoxicity tests as well as in experiments on activity of CELA W 524 against plant diseases, isolates of the following saprophytes and non-obligate parasites were employed: *Alternaria tenuis*, *Ascochyta pisi*, *Aspergillus niger*, *Botrytis cinerea*, *Cladosporium cucumerinum*, *Cladosporium fulvum*, *Colletotrichum lindemuthianum*, *Colletotrichum orbiculare*, *Fusarium culmorum*, *Fusarium oxysporum* f. *pisi*, *Fusarium roseum*, *Glomerella cingulata*, *Penicillium expansum* and *Stemphylium radicinum*; in addition, CELA W 524 was tested against some powdery mildew diseases caused by *Erysiphe*

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[2] See note on p. 44.

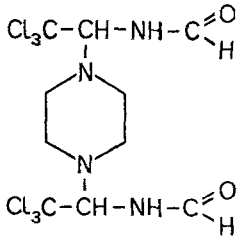

Fig. 1. Structural formula of N,N'-bis-(1-formamido-2,2,2-trichloroethyl)-piperazine (CELA W 524).
Fig. 1. Structuurformule van N,N'-bis-(1-formamido-2,2,2-trichloorethyl)-piperazine (CELA W 524).

graminis f. sp. *hordei* (barley), *Erysiphe pisi* (pea), and *Sphaerotheca fuliginea* (cucumber), and against bean rust, caused by *Uromyces appendiculatus*. In phytotoxicity tests and in experiments on activity of CELA W 524 against plant diseases the following test plants were used: barley (cvs 'Balder', 'Cambrinus'), bean (cvs 'Dubbele Witte z. dr.', 'Processor'), cucumber (cvs 'Lange Gele Tros', 'Spotvrije donkergroene'), pea (cvs 'Kelvedon Wonder', 'Mansholt', 'Rondo'), tobacco (cv. 'Samsun NN'), and tomato (cvs 'Ailsa Craig', 'Bonner Beste').

CELA W 524 was obtained from C. H. Boehringer Sohn in pure crystalline form and as emulsifiable concentrates of 10 and 20%, respectively; in control experiments, blanks of both concentrates were used, which instead of CELA W 524 contained 10 and 20% H_2O, respectively. For convenience' sake, the preparations employed are indicated as follows: B1 (10% CELA W 524; contains a nonionic emulsifier), B2 (blank of B1), B4 (20% CELA W 524; contains an anionic emulsifier) and B5 (blank of B4).[1] All concentrations of CELA W 524 given in the present article are based on the active ingredient.

Fungitoxicity test

Fungitoxicity of CELA W 524 was examined by various tests. First, the fungal isolates mentioned were grown in Czapek Dox liquid medium or on Czapek Dox agar, fortified with 1% malt extract, to which after heat sterilization CELA W 524 was added in various amounts thus giving a series of concentrations. Dry weights of mycelia (liquid media) or radial growth of colonies (agar media) served as criteria to estimate inhibitory effects. In other tests germination of conidia in droplets of Czapek Dox liquid medium on glass slides as well as measurement of germ-tube length was employed to estimate fungitoxic action. Finally, use was made of a thin layer chromatographic bioassay on silica gel plates (DC-Alufolie Kieselgel F 254, Merck) (Homans and Fuchs, 1970) to assess the inhibitory action of CELA W 524 on fungal growth.

Phytotoxicity tests

Tests for phytotoxicity were performed by spraying whole plants with aqueous solutions of CELA W 524; in other instances, whole plants or shoots were allowed to take up the fungicide from aqueous solutions via their root system or via the cut end of the stem, respectively. Control plants always received the blank of the emulsifiable concentrates used, in concentrations comparable to those employed in the treated plants.

[1] At the beginning of our investigations only the preliminary 10% emulsifiable concentrate (B1) was available. Because of its phytotoxicity it has been replaced meanwhile by the virtually non-phytotoxic 20% emulsifiable concentrate (B4). Results of experiments with B1 (and B2) are, in fact, only given for the sake of comparison; for practical use only the formulation B4 should be recommended.

Tests for activity against plant diseases

Activity of CELA W 524 against plant diseases was examined a. by spraying plants grown in sand, in garden soil or on Hoagland solution before or after inoculation with aqueous solutions of the fungicide, b. by applying CELA W 524 as a soil drench after inoculation, or c. by seed treatment. The systemic character of CELA W 524 was investigated by allowing whole plants to take up the fungicide from aqueous solutions via their root system during two days; then, they were inoculated and the fungicide solutions replaced by tap water. In the latter experiments, plants receiving the blanks served as controls.

All phytotoxicity tests as well as experiments on activity against plant diseases were carried out under greenhouse conditions or in temperature, light and relative humidity controlled climate rooms.

Results

Fungitoxicity tests

Table 1 summarizes the results of experiments on the fungitoxicity of the two formulations of CELA W 524 (B1, B4) as compared to their respective blanks (B2, B5), expressed as inhibition of growth on Czapek Dox-malt agar. From these results it is obvious that a relatively slight inhibition of growth by B1 and B4 is superimposed on a marked inhibition by the inert ingredients[1] themselves (B2, B5); only in the case of *Cladosporium cucumerinum* and *Ascochyta pisi*, and to a lesser extent in that of *Colletotrichum orbiculare*, *Glomerella cingulata* and *Aspergillus niger* the additional inhibition caused by CELA W 524 is quite distinct. No inhibition at all was found in the case of *Stemphylium radicinum*. The inhibition by CELA W 524 seems to be more marked when applied as B4 than as B1.

Using liquid media comparable results were obtained; here, however, the inhibitory effect of the inert ingredients appeared to be somewhat less pronounced, rendering the inhibitory activity of CELA W 524 (especially as B4) relatively more conspicuous, in particular in the case of *Aspergillus niger*. Growth of *Fusarium oxysporum* f. *pisi*, a fungus not tested on agar medium, was not markedly inhibited by CELA W 524.

From Table 2, which enumerates the data on inhibition of spore germination and germ tube elongation, it appears that CELA W 524 strongly inhibits spore germination of *Aspergillus niger* and *Cladosporium cucumerinum*, although in the latter fungus this effect is obscured somewhat in the case of B1 by the inhibitory activity of the inert ingredients present therein (compare B2). No inhibitory effect at all was observed in *Botrytis cinerea*, *Glomerella cingulata* and *Stemphylium radicinum*.

Regarding the effect on germ tube growth B1 and B4 exerted a distinct inhibitory action, in particular in *Aspergillus niger* and *Cladosporium cucumerinum* and also, although to a lesser extent, in *Ascochyta pisi*, *Botrytis cinerea* and *Glomerella cingulata*; in the case of *Stemphylium radicinum* the inhibition was slight to nil as compared to the inhibitory activity of the inert ingredients alone.

As shown in Fig. 2, the thin layer chromatographic bioassay revealed a very distinct inhibition of fungal growth at R_f-value 0.21 by 50 μg amounts (based on active ingre-

[1] The term 'inert ingredients' is used to indicate the total of emulsifier(s), solvent(s), etc. present in the formulations employed. Its use is not meant to imply lack of any biological activity of the inert ingredients.

Table 1. Fungitoxicity in vitro of B1, B2, B4, and B5, based on inhibition of growth (in diameter) after 3 days on Czapek Dox-malt agar.

Fungus tested	Substance tested	Concentrations tested (mg/l)[1]					
		100	50	25	10	5	2.5
Alternaria tenuis[2]	B1	+[3]	+	+	++	+++	+++
	B2	0	+	+	++	+++	+++
	B4	0	0	+	++	+++	+++
	B5	0	+	++	+++	+++	+++
Ascochyta pisi[2]	B1	0	0		+		+
	B2	0	0		+		++
	B4	0	0		+		++
	B5	0	+		+++		+++
Aspergillus niger[2]	B1	0	+	+	++	++	+++
	B2	0	+	++	++	+++	+++
	B4	0	0	+	+	++	+++
	B5	0	+	++	+++	+++	+++
Botrytis cinerea[2]	B1	0	0	+	++	+++	+++
	B2	0	0	+	++	++	+++
	B4	0	0	+	++	++	+++
	B5	0	+	+	++	+++	+++
Cladosporium cucumerinum	B1	0	0		+		++
	B2	0	0		++		+++
	B4	0	0		+		++
	B5	0	+		+++		+++
Colletotrichum lindemuthianum	B1	0	0		0		++
	B2	0	0		++		++
	B4	0	0		++		+++
	B5	0	0		+++		+++
Colletotrichum orbiculare	B1	0	0		0		+++
	B2	0	0		+++		+++
	B4	0	+		+++		+++
	B5	0	++		+++		+++
Glomerella cingulata	B1	0	0		++		++
	B2	0	0		++		+++
	B4	0	+		++		++
	B5	0	++		+++		+++
Stemphylium radicinum[2]	B1	0	++	++	++	+++	+++
	B2	0	0	++	+++	+++	+++
	B4	0	0	++	+++	+++	+++
	B5	0	0	+++	+++	+++	+++

[1] Based on active ingredient, as present in B1 and B4, respectively. Inert ingredients were present in concentrations of 900, 450, 225, 90, 45, and 22.5 mg/l, respectively, in the case of B1 and B2, and of 400, 200, 100, 40, 20, and 10 mg/l, respectively, in the case of B4 and B5.

[2] Inoculated with a 3 mm agar disc in the centre of the plate; in the other instances, inoculated with a droplet of a spore suspension in the centre of the plate.

[3] 0, +, ++, +++: less than 5%, 5%–33%, 34%–66% and 67%–100% of normal growth, respectively.

Tabel 1. In vitro fungitoxiciteit van B1, B2, B4 en B5, gebaseerd op remming van de groei (in diameter) na 3 dagen op Czapek Dox-moutagar.

Table 2. Fungitoxicity in vitro of B1, B2, B4, and B5, based on inhibition of germination of conidia and germ tube elongation; conidia were allowed to germinate in small droplets of Czapek Dox liquid medium on glass slides, which were kept in a humid chamber for 20 h.

Fungus tested	Substance tested	Spore germination (control = 100)			Germ tube length[1] (control = 100)		
		Concentrations tested (mg/l); see footnote Table 1					
		100	50	10	100	50	10
Ascochyta pisi	B1	100	100	100	50	56	52
	B2	100	100	100	66	61	80
	B4	14	100	100	13	34	46
	B5	63	100	100	24	74	108
Aspergillus niger	B1	0	1	35	–	(13)	51
	B2	100	100	100	77	75	95
	B4	0	0	2	–	–	(13)
	B5	100	100	100	87	106	111
Botrytis cinerea	B1	100	100	100	37	56	84
	B2	100	100	100	106	135	140
	B4	100	100	100	47	63	133
	B5	100	100	100	105	128	141
Cladosporium cucumerinum	B1	0	0	93	–	–	12
	B2	16	10	99	24	(28)	75
	B4	6	35	92	(10)	4	12
	B5	100	100	100	23	96	124
Glomerella cingulata	B1	100	100	100	37	64	91
	B2	100	100	100	108	89	121
	B4	100	100	100	55	84	83
	B5	100	100	100	131	133	139
Stemphylium radicinum	B1	100	100	100	32	40	58
	B2	100	100	100	42	46	50
	B4	100	100	100	89	96	111
	B5	100	100	100	115	98	104

[1] When % of germination was $\leq$ 10, data on germ tube length are given in parentheses.

Tabel 2. In vitro fungitoxiciteit van B1, B2, B4 en B5, gebaseerd op remming van de sporekieming en van de groei van de kiembuis; de sporekieming werd onderzocht in druppels van een vloeibaar Czapek Dox medium op objectglazen, na een verblijf van 20 uur in een vochtige omgeving.

dient) of B1 and B4 in *Botrytis cinerea*, *Cladosporium cucumerinum*, *Fusarium culmorum* and *Penicillium expansum*; a slight inhibition was observed in the case of *Ascochyta pisi*, *Aspergillus niger* and *Fusarium roseum*; growth of *Glomerella cingulata* was virtually not inhibited. In addition, much less pronounced zones of inhibition at several R_f-values could be observed in the case of B1 and B2 with *Cladosporium cucumerinum* and *Fusarium culmorum* whereas B4 and B5 produced distinct inhibition zones with R_f-value 0.00 in *Ascochyta pisi*, *Botrytis cinerea*, *Cladosporium cucumerinum*, *Fusarium roseum* and *Penicillium expansum*. Clearly, the latter inhibition of fungal growth by both B1 and B2 (at several R_f-values) and by B4 as well as B5 (at R_f-value 0.00) must be entirely due to (components of) the inert ingredients present. Fifty μg amounts of the active ingredient alone, dissolved in ethanol, caused a marked

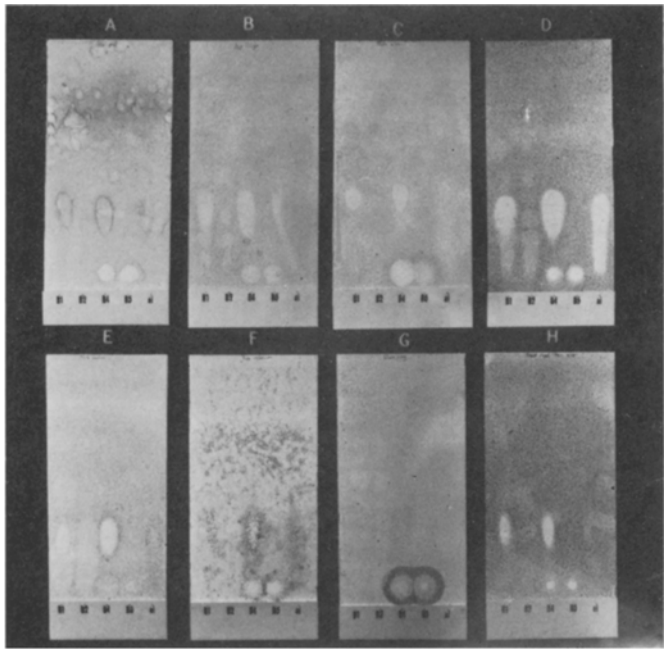

Fig. 2. Differential sensitivity for CELA W 524 of: (A) *Ascochyta pisi*; (B) *Aspergillus niger;* (C) *Botrytis cinerea;* (D) *Cladosporium cucumerinum;* (E) *Fusarium culmorum;* (F) *Fusarium roseum;* (G) *Glomerella cingulata;* (H) *Penicillium expansum.* The quantities spotted as B1, B4 and a.i. (active ingredient) contained or equalled 50 μg active ingredient.
Fig. 2. Uiteenlopende gevoeligheid voor CELA W 524 van: (A) Ascochyta pisi; (B) Aspergillus niger; (C) Botrytis cinerea; (D) Cladosporium cucumerinum; (E) Fusarium culmorum; (F) Fusarium roseum; (G) Glomerella cingulata; (H) Penicillium expansum. De gechromatografeerde hoeveelheden B1, B4 en a.i. (werkzaam bestanddeel) bevatten, respectievelijk kwamen overeen met, 50 μg CELA W 524.

zone of inhibition only in *Cladosporium cucumerinum*; in other bioassays even amounts as low as 5 μg produced a visible inhibition zone.

The seeming discrepancy between the experimental results with B1 and B4 versus the active ingredient alone in the case of *Botrytis cinerea*, *Fusarium culmorum* and *Penicillium expansum* might be explained by solubility characteristics of CELA W 524: comparison of different solvents, used in the preparation of solutions of CELA W 524, showed the size of the zone of inhibition to depend more or less on the nature of the solvent.

Phytotoxicity tests

In Table 3 data on the phytotoxicity of CELA W 524 have been summarized. A few general conclusions can be drawn. When sprayed B1 and B2, at high concentrations, caused phytotoxic symptoms; no symptoms were observed in the case of B4 and B5. When applied via the roots, again only B1 and B2 caused appreciable phytotoxicity, especially in the case of cucumber (see Table 4) and tobacco; further, marked inhibition of growth was observed in barley (Fig. 3). B4 and B5, on the other hand, were only slightly phytotoxic to tobacco at the highest concentrations tested. The phytotoxicity seems entirely due to the inert ingredients employed as can be deduced from a com-

Table 3. Phytotoxicity of CELA W 524.

Plant	Age[1]	Concentrations tested (mg/l)	Symptoms
Application by spraying (one spray); plants kept standing in Hoagland solution			
barley, cv. 'Cambrinus'	10 d[2]	8, 16, 31, 62, 125, 250, 500	B1: at > 250 mg/l: marginal leaf necrosis
bean, cv. 'Processor'	14 d[3]	idem	B1, B2: at > 250 mg/l: leaf vein necrosis
tomato, cv. 'Bonner Beste'	42 d[4]	idem	B1, B2: at > 250 mg/l: mild leaf spots on older leaves only
Application via the roots; plants kept standing in Hoagland solutions containing either B1, B2, B4, or B5			
barley, cv. 'Cambrinus'	10 d[2]	1.5, 3, 6, 12, 25, 50, 100	at > 12 mg/l: inhibition of plant growth and of water uptake; B1 and B2 gave much stronger effect than B4 and B5; some yellowing of leaves
bean, cv. 'Processor'	14 d[3]	idem	none
tobacco, cv. 'Samsun NN'	34 d[5]	idem	B1, B2: at > 6 mg/l: yellow leaf spots becoming dry with age B4, B5: at > 50 mg/l: idem
tomato, cv. 'Bonner Beste'	42 d[4]	idem	none
Application via cut stems; plants kept standing in Hoagland solutions containing either B1, B2, B4, or B5			
bean, cv. 'Processor'	14 d[3]	1.5, 3, 6, 12, 25, 50, 100	B1, B2: at > 12 mg/l: shrivelling of leaves B4, B5: no symptoms
tomato, cv. 'Bonner Beste'	42 d[4]	idem	B1, B2: at > 12 mg/l: severe shrivelling of leaves, collapse of stem B4, B5: at > 100 mg/l: some shrivelling of leaves

[1] At time of beginning of treatment; phytotoxic symptoms evaluated [2] 10 days, [3] 5 days, [4] 4 days and [5] 7 days after beginning of treatment.

Tabel 3. Fytotoxiciteit van CELA W524.

parison of data on phytotoxicity of CELA W 524 applied via the cut stem of bean and tomato plants: whereas B1 and B2 caused a complete collapse of the stems and, thus, severe shrivelling of leaves, B4 and B5-treated plants showed no or only very slight signs of phytotoxicity.

Phytotoxicity of B1 and B2 seemed to depend to a certain extent on age of the treated plants or plant parts, symptoms in many cases being much more severe in older leaves than in younger ones. Moreover, on spraying damage was found to be confined to the actually sprayed parts, newly developing sprouts remaining completely free of phytotoxic symptoms.

Tests for activity against plant diseases

Results on activity of CELA W 524 against plant diseases have been enumerated in Table 4. Spraying of barley (cv. 'Balder') and cucumber plants (cv. 'Lange Gele Tros',

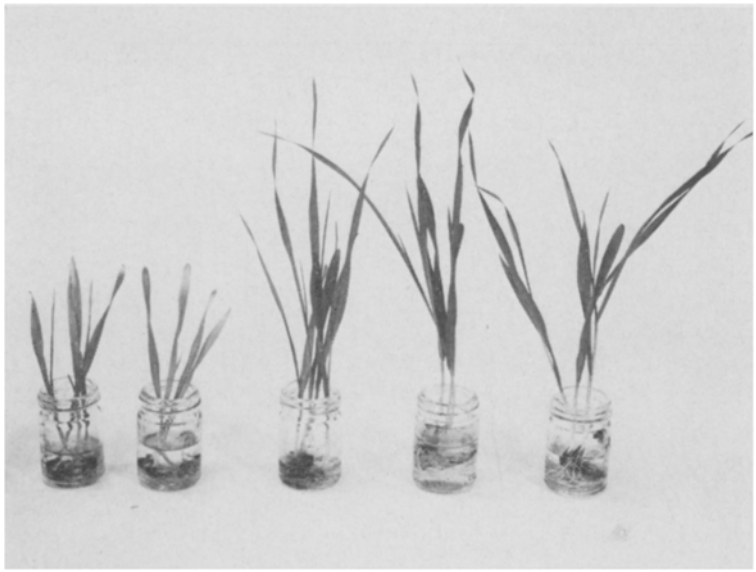

Fig. 3. Influence of different formulations of CELA W 524 on growth of barley plants (cv. 'Cambrinus') upon application via the roots; (from left to right) B1, B2, B4, B5 (all concentrations 100 mg/l) and control (untreated).

Fig. 3. Invloed van verschillende formuleringen van CELA W 524 op de groei van gerstplanten (cv. 'Cambrinus') na toediening via de wortels; (van links naar rechts) B1, B2, B4, B5 (alle concentraties 100 mg/l) en controle (onbehandeld).

'Spotvrije donkergroene') with B1 four times in a 48 h period before inoculation resulted in complete protection against barley and cucumber powdery mildew (*Erysiphe graminis* f. sp. *hordei*, *Sphaerotheca fuliginea*) at concentrations of 50 and 5 mg/l, respectively, without causing phytotoxic symptoms. No protection was obtained upon spraying of B1 against cucumber anthracnose (*Colletotrichum orbiculare*), and leaf spot in peas (*Ascochyta pisi*). Upon pre-infectional spraying with B4 of barley (cv. 'Cambrinus'), bean (cv. 'Dubbele Witte z. dr.'), cucumber (cv. 'Lange Gele Tros'), pea (cv. 'Mansholt') and tomato plants (cv. 'Ailsa Craig') comparable results were obtained, concentrations of 25 or even 5 mg/l being effective against barley powdery mildew (*Erysiphe graminis* f. sp. *hordei*), bean rust (*Uromyces appendiculatus*), cucumber powdery mildew (*Sphaerotheca fuliginea*) as well as cucumber scab (*Cladosporium cucumerinum*), pea powdery mildew (*Erysiphe pisi*) and tomato leaf mould (*Cladosporium fulvum*). Only at the highest concentration employed (125 mg/l) a distinct, although not complete protection was observed against leaf spot in pea plants. Post-infectional spraying with B4 resulted in a less effective protection of plants in most plant-parasite combinations examined; yet, complete protection against the three mildew diseases studied and against leaf mould in tomato plants was observed at concentrations of 25 and 125 mg/l. Neither application of CELA W 524 as a soil drench nor seed treatment was successful in protecting pea plants against Fusarium wilt (*Fusarium oxysporum* f. *pisi*).

CELA W 524, in both formulations (B1, B4), displayed systemic activity protecting barley plants (cv. 'Cambrinus') against powdery mildew at all concentrations tested.

Table 4. Activity of CELA W 524 against plant diseases.

Plant	Age[1]	Disease	Concentrations tested (in mg/l)	Time of evaluation
Application (of formulation B1 only) by spraying 4 times in 48 h period before inoculation; plants grown in garden soil				
barley, cv. 'Balder'	?	powdery mildew (*E. graminis* f. sp. *hordei*)	0.5, 5, 50	12 days p.i.[2]
cucumber, cv. 'Lange Gele Tros'	?	powdery mildew (*Sph. fuliginea*)	0.5, 5	10 days p.i.
cucumber, cv. 'Spotvrije donkergroene'	3 w	powdery mildew (*Sph. fuliginea*)	0.5, 5, 50	9 days p.i.
cucumber, cv. 'Spotvrije donkergroene'	3 w	anthracnose (*C. orbiculare*)	0.5, 5, 50	9 days p.i.
pea, cv. 'Kelvedon Wonder'	3 w	leaf spot (*A. pisi*)	5, 10, 20, 30	10 days p.i.
Application (of formulation B4 only) by spraying 4 times in 48 h period before inoculation; plants kept standing i				
barley, cv. 'Cambrinus'	9 d	powdery mildew (*E. graminis* f. sp. *hordei*)	5, 25, 125	9 days p.i.
bean, cv. 'Dubbele Witte z. dr.'	13 d	bean rust (*U. appendiculatus*)	5, 25, 125	12 days p.i.
cucumber, cv. 'Lange Gele Tros'	8 d	powdery mildew (*Sph. fuliginea*)	5, 25, 125	9 days p.i.
cucumber, cv. 'Lange Gele Tros'	8 d	cucumber scab (*C. cucumerinum*)	5, 25, 125	9 days p.i.
pea, cv. 'Mansholt'	16 d	leaf spot (*A. pisi*)	5, 25, 125	12 days p.i.
pea, cv. 'Mansholt'	16 d	powdery mildew (*E. pisi*)	5, 25, 125	7 days p.i.
tomato, cv. 'Ailsa Craig'	27 d	tomato leaf mould (*C. fulvum*)	5, 25, 125	12 days p.i.
Application (of formulation B4 only) by spraying 3 times, 1, 5 and 9 days after inoculation; plants kept standing in				
barley, cv. 'Cambrinus'	9 d	powdery mildew (*E. graminis* f. sp. *hordei*)	5, 25, 125	9 days p.i.
bean, cv. 'Dubbele Witte z. dr.'	9 d	bean rust (*U. appendiculatus*)	5, 25, 125	12 days p.i.
cucumber, cv. 'Lange Gele Tros'	8 d	powdery mildew (*Sph. fuliginea*)	5, 25, 125	9 days p.i.
cucumber, cv. 'Lange Gele Tros'	8 d	cucumber scab (*C. cucumerinum*)	5, 25, 125	9 days p.i.[3]
pea, cv. 'Mansholt'	16 d	leaf spot (*A. pisi*)	5, 25, 125	9 days p.i.
pea, cv. 'Mansholt'	16 d	powdery mildew (*E. pisi*)	5, 25, 125	9 days p.i.
tomato, cv. 'Ailsa Craig'	27 d	tomato leaf mould (*C. fulvum*)	5, 25, 125	12 days p.i.
Application (of formulation B1 only) as soil drench 3 times in 3 d period after inoculation; plants grown in sand				
pea, cv. 'Rondo'	–	pea wilt (*F. oxysporum* f. *pisi*)	10 mg/50 seeds daily for 3 d	(35 days p.i.)
Application (of formulation B1 only) as seed treatment; plants grown in sand				
pea, cv. 'Rondo'	–	pea wilt (*F. oxysporum* f. *pisi*)	1 mg/seed	(35 days p.i.)
Application via the roots; plants kept standing in aqueous solutions of B1, B2, B4, and B5 for 2 days; then inoculated				
barley, cv. 'Cambrinus'	13 d	powdery mildew (*E. graminis* f. sp. *hordei*)	1.5, 3, 6, 12, 25, 50	12 days p.i.
cucumber, cv. 'Lange Gele Tros'	7 d	cucumber scab (*C. cucumerinum*)	1.5, 3, 6, 12, 25, 50	7 days p.i.
cucumber, cv. 'Lange Gele Tros'	7 d	powdery mildew (*Sph. fuliginea*)	1.5, 3, 6, 12, 25, 50	12 days p.i.
pea, cv. 'Mansholt'	?	leaf spot (*A. pisi*)	25, 50, 100, 200	4 days p.i.

[1] At time of inoculation
[2] p.i., after inoculation
[3] Sprayed twice only, 1 and 5 days after inoculation

Table 4. Werkzaamheid van CELA W 524 tegen planteziekten.

Effectiveness
moderate and slight infection (3–4 % of leaf area) at 0.5 and 5 mg/l, respectively; no symptoms at 50 mg/l
no symptoms at 0.5 and 5 mg/l
no symptoms at 0.5 and 5 mg/l; phytotoxic at 50 mg/l
no appreciable protection
no protection at all
Hoagland solution
no symptoms at all concentrations tested
slight infection at 5 mg/l; no symptoms at 25 and 125 mg/l
no symptoms at all concentrations tested
moderate and very slight infection at 5 and 25 mg/l, respectively; no symptoms at 125 mg/l
no protection at 5 mg/l; slight to distinct suppression of symptoms at 25 and 125 mg/l
slight infection at 5 mg/l; no symptoms at 25 and 125 mg/l
almost complete protection at 5 mg/l; no symptoms at 25 and 125 mg/l
Hoagland solution
almost no protection at 5 mg/l; no symptoms at 25 and 125 mg/l
no protection at 5 and 25 mg/l; moderate infection at 125 mg/l
moderate infection at 5 mg/l; no symptoms at 25 and 125 mg/l
no protection at 5 and 25 mg/l; slight suppression of symptoms at 125 mg/l
hardly any effect at any concentration tested
almost complete protection at 5 mg/l; no symptoms at 25 and 125 mg/l
considerable protection at 5 mg/l; no symptoms at 25 and 125 mg/l
after initial distinct retardation of symptom expression progressive wilting and dying of plants
as above, but protective effect even smaller
and transferred to tap water
B1, B4: complete protection at all concentrations tested (B1 – as well as B2 – were phytotoxic at $>$ 25 mg/l)
B1: no protection at non-phytotoxic concentrations ($<$ 12 mg/l) B4: complete protection at 25 and 50 mg/l
B1: complete protection at 6 mg/l; phytotoxic at higher concentrations B4: slight infection at 12 and 25 mg/l; complete protection at 50 mg/l
no protection at all concentrations tested

Fig. 4. Protection of cucumber plants (cv. 'Lange Gele Tros') against cucumber scab (*Cladosporium cucumerinum*) by CELA W 524; plants were allowed to take up the fungicide from aqueous solutions of B4, containing 25 mg a.i./l, for a period of 2 days before inoculation.
Fig. 4. Bescherming van komkommerplanten (cv. 'Lange Gele Tros') tegen vruchtvuur (Cladosporium cucumerinum) door CELA W 524 na opname van het fungicide uit een waterige oplossing van B4 (25 mg/l), gedurende 2 dagen vóór inoculatie.

Cucumber plants (cv. 'Lange Gele Tros') were protected against powdery mildew at concentrations of as low as 6 mg/l in the case of B1 and of 50 mg/l in the case of B4, and against scab (Fig. 4) at concentrations of 25 mg/l in the case of B4. It proved, however, not to be systemically active against leaf spot in peas.

Whenever examined, the blanks (B2, B5) displayed no protective activity at all; thus, protection of plants by B1 and B4 must be exclusively ascribed to the presence of CELA W 524.

Discussion

Schicke and Veen (1969) found CELA W 524 particularly active against mildews, rusts and apple scab. Their results indicate that root treatment is more effective than foliar treatment in protecting rye against powdery mildew and wheat against leaf rust. With root treatments concentrations in the order of magnitude of 5 mg/l were found to suppress leaf symptoms to less than 2% of control plants, whereas concentrations needed to obtain similar effects upon foliar treatment were about 20 times as high. Our results, although not allowing an exact comparison of different treatments, do not affirm the impression that, at comparable concentrations, root treatment provides a more effective means of protecting plants than foliar sprays: against barley powdery mildew root treatment proved to be more effective, but against cucumber powdery mildew foliar treatment provided better results. In this connection, it is worth noting, that in order to prevent phytotoxic damage in the spray trials with

formulation B1 relatively low concentrations had to be employed throughout; experiments with the virtually non-phytotoxic formulation B4, which, therefore, could be used at higher concentrations, provided much better results.

In contrast to the experiences of Schicke and Veen (1969) with *Erysiphe graminis* we found that CELA W 524 markedly inhibited germination of conidia of some fungi, as f.i. *Aspergillus niger* and *Cladosporium cucumerinum*; in addition, mycelial growth was quite distinctly inhibited by CELA W 524 in a series of pathogenic and non-pathogenic fungi. Especially, however, when CELA W 524 was tested using a thin layer chromatographic bioassay (Homans and Fuchs, 1970), the substance was found to exert a direct fungitoxic action on spore germination and fungal growth. Schicke and Veen's statement (1969) that, in the case of *Erysiphe graminis*, CELA W 524 seems to need the living plant to become effective could suggest, that CELA W 524 should be transformed only within the living plant into an effective fungicide. Our results do not corroborate this hypothesis; other results (Fuchs et al., 1970) are even suggestive of the fact, that in plants CELA W 524 is readily converted to non-fungitoxic metabolites. A more detailed report on the latter results will be published elsewhere.

Acknowledgment

The skilful assistance of Mrs. M. Viets-Verweij is gratefully acknowledged.

Samenvatting

In vitro en in vivo activiteiten van een nieuw systemisch fungicide, N,N'-bis-(1-formamido-2,2,2-trichloorethyl)-piperazine (CELA W 524)

Het nieuwe systemische fungicide CELA W 524 (C. H. Boehringer Sohn, Ingelheim am Rhein, Duitsland) bleek een matige tot duidelijke fungitoxische werking in vitro te vertonen tegenover verschillende pathogene en niet-pathogene schimmels. Eén van de beschikbare formuleringen bleek vrij sterk fytotoxisch, de andere was nagenoeg niet fytotoxisch. Bespuiting vóór inoculatie met de niet-fytotoxische formulering resulteerde in volledige bescherming van gerst, bonen, komkommers, erwten en tomaten tegen respectievelijk gerstemeeldauw, boneroest, komkommermeeldauw en vruchtvuur, erwtemeeldauw en bladvlekkenziekte bij tomaat. Enige onderdrukking van ziektesymptomen trad ook op bij erwten, geïnoculeerd met *Ascochyta pisi*, tenminste, wanneer hoge concentraties van CELA W 524 werden gebruikt. Bij bespuiting na inoculatie was het effect geringer, hoewel meeldauwziekten en bladvlekkenziekte bij tomaat toch doeltreffend bestreden werden. Toegediend via de wortels bleek CELA W 524 systemisch actief; het beschermde aldus gerst tegen meeldauw en komkommers tegen meeldauw en vruchtvuur.

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