Neth. J. Pl. Path. 72 (1966): 213-221

ACTIVITY AGAINST RUST AND POWDERY MILDEW OF SOME *PARA* PHENYLENEDIAMINES AND RELATED COMPOUNDS[1,2]

Activiteit tegen roest en meeldauw van enige para*fenyleendiamines en verwante verbindingen*

G. J. NIEMANN[3] and J. DEKKER
T.N.O. Research Unit for Internal Therapy of Plants
Institute for Organic Chemistry T.N.O., Utrecht
Laboratory of Phytopathology, Wageningen

Twenty-five *para* phenylenediamines and related compounds were tested for activity against powdery mildew on cucumber and against rust on bean. Some remarkable changes in activity resulted from minor changes in the chemical structure of the compounds. It is suggested that the fungicidal activity of the phenylenediamines is correlated with the polarographic halfwave potentials. This might indicate that one of the oxidation products, probably the semiquinone ion, is responsible for activity.

INTRODUCTION

In the literature fungicidal activity had been reported for several *para* phenylenediamines. For instance, *N,N*-dimethyl-*p*-phenylenediamine (WEAVER *et al.*, 1959), *N,N*-di-*sec*-octyl-*p*-phenylenediamine (SMOCK, 1957) and *N,N*-diphenyl-*p*-phenylenediamine (STEVENSON & BLAKE, 1961) have been found to be active against a number of wood-destroying fungi or against apple scald.

Our attention was drawn to the antifungal activity of phenylenediamines by the anti-mildew properties of 3-methyl-N^1,N^1-diethyl-*p*-phenylenediamine in screening experiments using *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll. on cucumber. In preliminary tests this compound appeared to be rather effective on leaf application, though when applied to the roots it was very phytotoxic.

It was decided to investigate a series of substituted *para* phenylenediamines for activity against *S. fuliginea* on cucumber. In the course of this work a fairly high activity of some of the phenylenediamines against *Uromyces appendiculatus* (Pers.) Ung. on French dwarf bean was found, so tests with this obligate parasite were also included.

MATERIALS AND METHODS

Some of the compounds used were commercially available. The greater part of the phenylenediamines, however, had to be synthesized for this work. Syntheses and physical data of the compounds have been described elsewhere (NIEMANN, 1964).

For the biological tests the compounds were dissolved or suspended in water, occasionally with the aid of a few drops of ethanol. The pH of the solution or suspension was brought to about 6.5 by titration with either aqueous sodium hydroxide or hydrochloric acid.

[1] Accepted for publication 15 February, 1966.
[2] Part of this work was described in the doctoral dissertation of the first author.
[3] Present address: Botanical Laboratory, University of Utrecht, Utrecht.

Fungicidal activity against *S. fuliginea* on cucumber, variety 'Lange gele tros', was evaluated both as a leaf spray and in a leaf-disc test. In a root application test the compounds were found to be too phytotoxic at concentrations which were active against powdery mildew. In the leaf spray test plants were sprayed twice on the first and second day after inoculation with a 1000 ppm solution or suspension of the compound to be examined. In the leaf-disc test seven discs, cut from cucumber leaves, were allowed to float in an open petri dish on a 100 ppm solution or suspension of the compound to be examined. More extensive descriptions of the test methods have been given earlier (DEKKER, 1961, 1963; NIEMANN & DEKKER, 1966).

Activity against *U. appendiculatus* on French dwarf bean cv. 'Dubbele witte zonder draad' was evaluated in a leaf-disc test which was described by DEKKER (1962). The concentration of the compounds used in this test was 6.25 ppm.

In the leaf spray test powdery mildew development was assessed about fourteen days after inoculation; in both leaf-disc tests development of powdery mildew and rust was assessed about eight days after inoculation. Fungal development was expressed in + and – symbols. For *S. fuliginea* on cucumber +++ was used when the leaves or discs were completely free from powdery mildew; ++ indicates about 0–10%; + about 10–30%; ± about 30–50% and – more than 50% coverage of the leaf surface with powdery mildew. For *U. appendiculatus* on French dwarf bean the pustules were counted. Here +++ was used when the discs were completely free from rust; ++ indicates about 0–10%; + about 10–30%; ± about 30–50% and – more than 50% of the rust development on the control discs. On the control discs an average of 45 pustules per disc was found.

Phytotoxicity was noted in the symbols:

tt = very toxic, the plants or discs being dead or almost dead,

t = toxicity symptoms such as necrotic spots, chlorosis, growth reduction, browning of the leaf edge *etc.*,

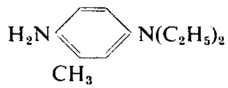
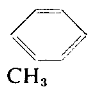
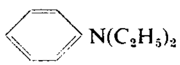
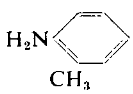
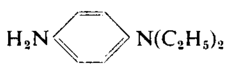
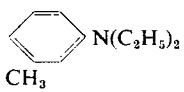
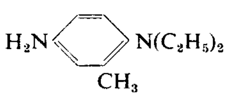
s = symptoms of slight toxicity, slight browning of the leaf edge, slight yellow discolouration *etc.*

RESULTS

Table 1 shows the influence of structural variations in 3-methyl-N^1,N^1-diethyl-*p*-phenylenediamine (1). Anti-mildew activity seems to be restricted to the phenylenediamine structure. Activity against rust, however, is also found for the anilines (2 and 4). Movement of a methyl group from the 3-position to the 2-position in the ring of *N*,*N*-diethyl-*p*-phenylenediamine has a striking effect. Whereas 3-methyl-N^1,N^1-diethyl-*p*-phenylenediamine (1) was quite active, 2-methyl-*N*,*N*-diethyl-*p*-phenylenediamine (8) appeared completely inactive against rust as well as against powdery mildew. The different activities of the compounds 1 and 8 are shown in Fig. 1.

In Table 2 the effect of substitution of the amino groups in *p*-phenylenediamine on antifungal activity is shown. It appears that whereas for anti-rust activity the phenylenediamine molecule itself (9) is sufficient, in the case of powdery mildew some substituents are required. The influence of replacement of methyl groups by ethyl is quite marked for anti-mildew activity (compare 11 and 6; 12 and 13). Remarkably *N*,*N*-diphenyl-*p*-phenylenediamine (16) is inactive in all cases, whereas *N*-phenyl-*p*-phenylenediamine (14) is quite active.

TABLE 1. Antifungal action of 3-methyl-N^1,N^1-diethyl-*p*-phenylenediamine and related compounds.
Fungicide werking van 3-methyl-N^1,N^1*-diethyl-*p*-fenyleendiamine en verwante verbindingen.*

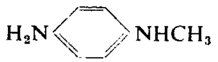
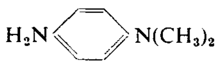
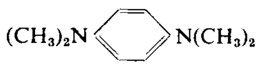
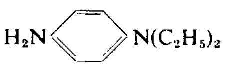
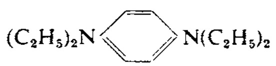
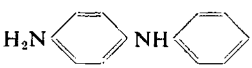
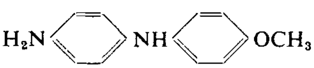
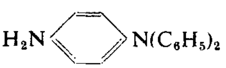
No.	Structure	*U. appendiculatus* leaf-disc test (6.25 ppm)	*S. fuliginea*	
			leaf-disc test (100 ppm)	leaf-spray test (1000 ppm)
1		++	tt[1]	±s
2		+	⁻s	–
3		⁻s	–	–
4		±s	–	–
5		–	⁻s	–
6		++	tt[1]	±s
7		⁻s	–	–
8		⁻s	tt[1]	⁻s

[1] inactive at non-phytotoxic concentrations

In Table 3 some compounds containing a substituent in the phenyl ring are summarized. As for the *p*-phenylenediamines in Table 2, in the structural variants of compound 1 lengthening of the alkyl substituent at the amino group is accompanied by an increase in anti-mildew activity in the leaf-disc test (18, 1 and 20). In the leaf-spray test this effect was less evident.

When comparing activities of the compounds in Table 3 with those of the compounds in Table 2 it appears that this introduction of a methyl substituent

TABLE 2. Antifungal action of some *p*-phenylenediamines substituted at amino groups.
Fungicide werking van enkele p-*fenyleendiamines, gesubstitueerd aan de amino-groepen.*

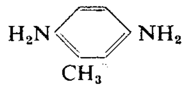
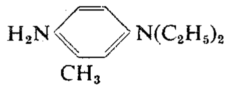
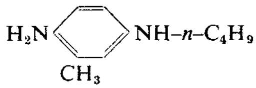
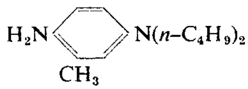
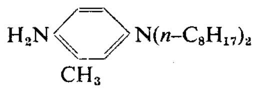
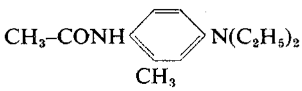
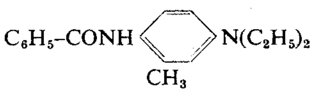
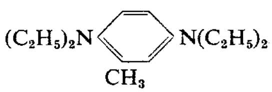
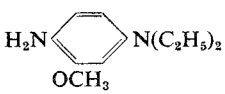
No.	Structure	*U. appendiculatus* leaf-disc test (6.25 ppm)	*S. fuliginea* leaf-disc test (100ppm)	*S. fuliginea* leaf-spray test (1000 ppm)
9		++	–	–
10		+s	–s	–
11		++	–	–
12		++	tt[1]	±s
6		++	+t	±
13		++s	tt[1]	++s
14		++	tt[2]	++
15		++	tt[2]	±
16		–	–t	–

[1] inactive at non-phytotoxic concentrations
[2] slightly active at non-phytotoxic concentrations

in the 3-position in general causes an increase in anti-mildew activity (compare 17, 18 and 1 with 9, 11 and 16). For compound 13, however, an almost total loss of anti-mildew activity was observed upon introduction of the methyl substituent (24).

Acetylation (22) or benzoylation (23) of the free amino group rendered 3-methyl-N^1,N^1-diethyl-*p*-phenylenediamine (1) totally inactive against both rust and mildew development.

TABLE 3. Antifungal action of *p*-phenylenediamines substituted in the phenyl ring.
Fungicide werking van in de ring gesubstitueerde p-*fenyleendiamines.*

No.	Structure	*U. appendiculatus* leaf-disc test (6.25 ppm)	*S. fuliginea* leaf-disc test (100 ppm)	*S. fuliginea* leaf-spray test (1000 ppm)
17		++	±s	±s
18		++	±s	±
1		++	+t	+
19		++	+t	±
20		++	++t	±
21		–	+s	+
22		–	–	–s
23		–	–	–
24		++	tt[1]	±s
25		±s	tt[2]	++

[1] inactive at non-phytotoxic concentrations [2] 25 ppm: ±

DISCUSSION

At first sight the results obtained offer no clear structure-activity relationships. The first question which arises is whether a similar mode of action may be assumed for both anti-rust and anti-mildew activity. A survey of the Tables shows that all compounds active against powdery mildew are active against rust as well, with one exception (compound 21). The reverse, however, is not true. Several compounds active against rust are inactive against powdery mildew. Of course, we have to bear in mind that the tests were carried out with very different concentrations. These concentrations were chosen in such a way that less than 100% activity was reached in order to obtain results which could be used to detect structure-activity relationships.

The most striking effects were obtained when the methyl substituent in methyl-N^1,N^1-diethyl-*p*-phenylenediamine was shifted from place 3 to 2 (compounds 1 and 8), or when the free amino group of compound 1 was blocked by acetylation or benzoylation (22 and 23), or when a second phenyl substituent was introduced in *N*-phenyl-*p*-phenylenediamine (14 to 16). In all cases active compounds were rendered totally inactive in both anti-rust and anti-mildew activity. This may lend support to the view that a similar mode of action is involved. It seems unlikely that lack of transportability can explain the inactivity of these compounds.

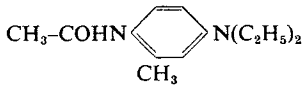
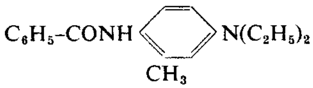
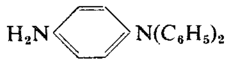
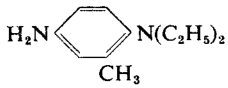
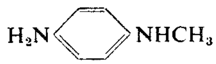
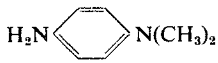
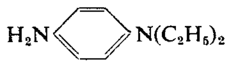
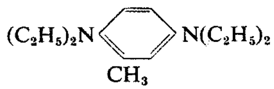
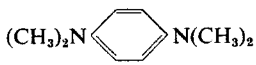
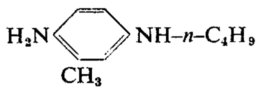
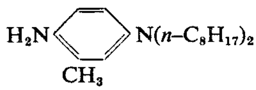
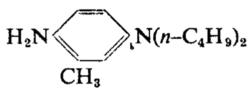
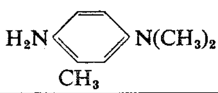
The effect of the methyl group placed *ortho* with respect to the diethylamino group in compounds 8 and 24 as compared with 6 and 13 (no methyl) or with 1 (methyl in *meta* position) may indicate that free rotation of the diethylamino group is important for activity. Free rotation is prevented by an *ortho* methyl substituent. Thus, steric effects may be responsible for the differences in activity found. Now *para* phenylenediamines are easily oxidized, which oxidation generally involves two steps. The primary oxidation product is a semiquinone ion which is stabilized by resonance. On further oxidation quinone-diimines may be formed which are generally unstable (MICHAELIS *et al.*, 1939). For effective resonance in the semiquinone ion the three substituents at the nitrogen atom and the latter must lie in one plane. If this configuration is sterically prevented the formation of the semiquinone will be hampered and oxidation becomes difficult.

A measure for oxidizability of the compounds can be found in the polarographic half-wave potentials ($E^1/_2$). The greater the tendency to release an electron, the more negative is the half-wave potential (European Convention).

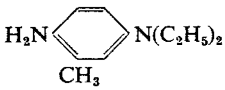
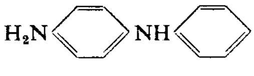
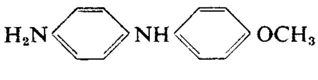
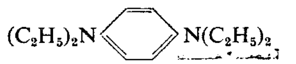
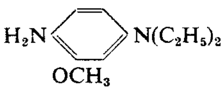
Since oxidizability is governed in part by steric effects, half-wave potentials may be used to obtain information regarding the steric configuration of the molecule. Thus it was tempting to compare the antifungal activity of the compounds with their half-wave potentials. In Table 4 the compounds have been listed according to decreasing half-wave potentials. Arguments for the listed order can be found elsewhere (NIEMANN, 1964). The compounds 9 and 17 were not included in Table 4 since these compounds are readily oxidized to the diimines which are extremely unstable in aqueous solution (MICHAELIS, 1939).

An inspection of Table 4 reveals that the compounds with the highest half-wave potentials (22, 23, 16 and 8) are inactive in all respects. Anti-rust activity starts at higher potentials than anti-mildew activity. In the leaf spray test a decrease in half-wave potential is accompanied by an increase in activity. As usual some exceptions are present as well, the general line, however, indeed

TABLE 4. Antifungal action of *p*-phenylenediamines, arranged according to decreasing half-wave potentials.
Fungicide werking van p-*fenyleendiamines, gerangschikt volgens afnemende halfwaarde-potentiaal.*

No.	Structure	*U. appendiculatus* leaf-disc test (6.25 ppm)	*S. fuliginea* leaf-disc test (100 ppm)	*S. fuliginea* leaf-spray test (1000 ppm)
22		–	–	$^{-}$s
23		–	–	–
16		–	$^{-}$t	–
8		$^{-}$s	tt	$^{-}$s
10		$^{+}$s	$^{-}$s	–
11		++	–	–
6		++	$^{+}$t[1]	$^{\pm}$s
24		++	tt	$^{\pm}$s
12		++	tt	$^{\pm}$s
19		++	$^{+}$t	±
21		–	$^{+}$s	+
20		++	$^{++}$t	±
18		++	$^{\pm}$s	±

[1] in other tests: tt

No.	Structure	*U. appendiculatus* leaf-disc test (6.25 ppm)	*S. fuliginea*	
			leaf-disc test (100 ppm)	leaf-spray test (1000 ppm)
1		++	+t[1]	+s
14		++	tt	++
15		++	tt	±
13		++s	tt	++s
25		±s	tt	++

seems to suggest a direct correlation between half-wave potential and activity. Such a correlation, of course, does not necessarily indicate that oxidation of the compounds is involved in antifungal activity. It seems very probable, however, that this is yet the case. Further evidence for the role of oxidation in the mode of action of *p*-phenylenediamines was obtained with 3-methoxy-N^1,N^1- diethyl-*p*-phenylenediamines (25); in the leaf-disc test with *S. fuliginea* the activity of this compound could be antagonized with reducing agents such as ascorbic acid and cysteine. In the rust leaf-disc test with the compounds 1, 13 and 25, however, no effects or only very slight ones were obtained with these reducing agents.

If oxidation is involved it is not certain whether the oxidation reaction itself or one or more of the oxidation products are responsible for activity. Since powdery mildews are rather sensitive to the action of quinones (NIEMANN, 1964) formation of such products might also offer an explanation for the activity of the phenylenediamines against powdery mildew and possibly against rust and other fungi as well. However, the very slight anti-mildew activity or inactivity of readily oxidizable compounds such as *p*-phenylenediamine (9) and methyl-*p*-phenylenediamine (17) partly contradicts this view. Furthermore, *p*-benzoquinone appeared almost inactive in both mildew tests. Thus, if indeed one of the oxidized forms of the *p*-phenylenediamines is responsible for activity, it seems logical to attribute this activity to the radical intermediate.

SAMENVATTING

Een vijfentwintigtal *para*fenyleendiamines en verwante verbindingen werd onderzocht op activiteit tegen komkommermeeldauw en boneroest. Zeer kleine wijzigingen in de chemische structuur van de verbindingen veroorzaakten grote veranderingen in activiteit (Fig. 1; Tabel 1, 2 en 3). Er wordt verondersteld dat er een verband is tussen de activiteit van de *para*fenyleendiamines en hun polarografische halfwaarde-potentiaal (Tabel 4). Dit zou erop kunnen wijzen dat de activiteit van *para*fenyleendiamines moet worden toegeschreven aan de vorming van oxydatie-produkten en wel waarschijnlijk aan het optreden van het semichinon-ion.

ACKNOWLEDGEMENTS

The authors are indebted to Mr. G. VAN DE VELDE for the preparation of the greater part of the compounds, and to Miss A. L. HOMANS for the execution of the biological tests.

REFERENCES

DEKKER, J., – 1961. Systemic activity of procaine hydrochloride on powdery mildew. Tijdschr. PlZiekt. 67:25–27.

DEKKER, J., – 1962. Systemic control of powdery mildew by 6-azauracil and some other purine and pyrimidine derivates. Meded. LandbHogesch. OpzoekStns Gent 27:1214–1221.

DEKKER, J., – 1963. Effect of kinetin on powdery mildew. Nature, Lond. 197:1027–1028.

MICHAELIS, L., M. P. SCHUBERT & S. GRANICK, – 1939. The free radicals of the type of Wurster's salts. J. Am. chem. Soc. 61:1981–1992.

NIEMANN, G. J., – 1964. Investigations on the chemical control of powdery mildew. Relationship between structure and activity of procaine and analogous compounds and of some paraphenylenediamines. Dissertation, Utrecht.

NIEMANN, G. J. & J. DEKKER, – 1966. Evaluation of the activity of a number of procaine derivatives and analogous compounds against powdery mildew (Sphaerotheca fuliginea) on cucumber. Ann. appl. Biol. 57:53–58.

SMOCK, R. M., – 1957. A comparison of treatments for control of the apple scald disease. Proc. Am. Soc. hort. Sci 69:91–100.

STEVENSON, C. D. & J. R. BLAKE, – 1961. Investigations into the control of superficial scald in coolstored Queensland grown Granny Smith apples by chemical means. Qd. J. agr. Sci. 18:293–314.

WEAVER, J. W., E. B. JEROSKI & I. S. GOLDSTEIN, – 1959. Toxicity of dyes and related compounds to wood-destroying fungi. Appl. Microbiol. 7:145–149.

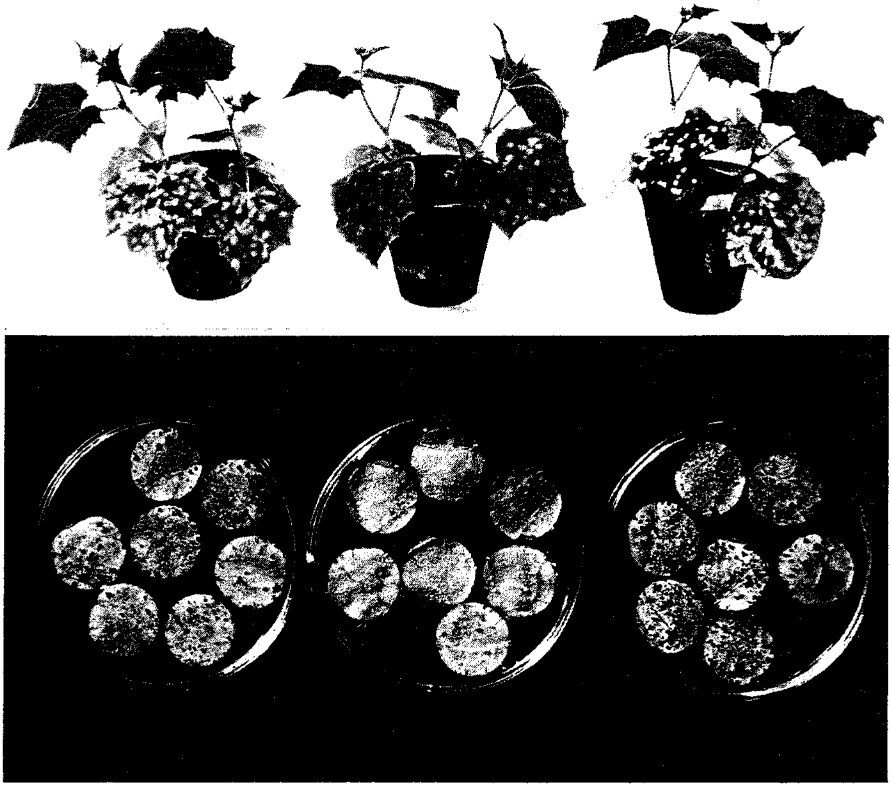
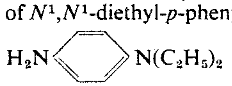


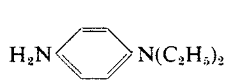
FIG. 1. Effect of the place of the methyl substituent on the activity of methyl-*N*,*N*,diethyl-*p*-phenylenediamine.

A. Powdery mildew on cucumber; leaves sprayed twice with water (left), with a 1000 ppm solution of the 3-methyl substituent (middle) and of the 2-methyl substituent (right) of N^1,N^1-diethyl-*p*-phenylenediamine.

B. Rust on French dwarf beans; leaf discs floating on water (left), on a 6.25 ppm solution of the 3-methyl substituent (middle) and of the 2-methyl substituent (right) of N^1,N^1-diethyl-*p*-phenylenediamine.



3-methyl-N^1,N^1-diethyl-*p*-phenylenediamine (no 1)



2-methyl-N^1,N^1-diethyl-*p*-phenylenediamine (no 8)

*Invloed van de plaats van de methyl-substituent op de activiteit van methyl-*N,N-*diethyl-*p-*fenyleendiamine.*

*A. Komkommermeeldauw; bladeren tweemaal bespoten met water (links), met een 1000 ppm op lossing van de 3-methyl-substituent (midden) en van de 2-methyl-substituent (rechts) van N^1,N^1-diethyl-*p-*fenyleendiamine.*

B. Roest op stambonen; bladschijfjes drijvend op water (links), op een 6.25 ppm oplossing van de 3-methyl-substituent (midden) en van de 2-methyl-substituent (rechts) van N,N-*diethyl-*p-*fenyleendiamine.*