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Algemene Bijdragen

THE MARSSONINA DISEASE OF POPLAR
*DE MARSSONINA-ZIEKTE VAN DE POPULIER**)

2. Inoculation experiments on leaf discs with ascospores and conidia
Inoculatieproeven op bladschijfjes met ascosporen en conidiën

[172.8 :416.15]

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SAMENVATTING

In deze tweede bijdrage over de Marssonina *bladvlekkenschimmel van de populier wordt bericht over inoculaties, die werden uitgevoerd op bladschijfjes van verschillende populierenklonen.*

Inoculaties, verricht met ascosporen en conidiën van „stippen" afkomstig van Aigeiros populieren infecteren alleen verwante klonen, alsmede Populus candicans *Ait. (sectie* Tacamahaca *Spach); ze geven geen infectie van* Populus alba *L. (series I. II en III). Inoculaties met ascosporen en conidiën van* Populus alba *infecteren alleen* Populus alba*; geen Aigeiros populieren (series I, II en III). Uit deze proeven blijkt bovendien dat infectie van boven- zowel als onderzijde van hetzelfde blad mogelijk is (series I t/m IV). Door een aantal klonen van Aigeiros te inoculeren met conidiën van „stippen" werd een indruk verkregen ten aanzien van hun gevoeligheid. Hieruit blijkt dat de klonen Serotina en Heidemij het hevigst worden aangetast, terwijl NL 925 de minst gevoelige is (serie IV). Inoculaties op bladschijfjes met conidiën van „stippen" produceren steeds weer „stippen"; inoculaties met conidiën afkomstig van „vlekken" wederom „vlekken" (serie VI). De afmetingen van de conidiën van beide typen bladvlekken zijn respectievelijk 9 - 17 × 4 - 7 μ en 17 - 24 × 6 - 11 μ, hetgeen hier alleen reeds wijst op het voorkomen van minstens twee verschillende vormen van Marssonina bij populieren van de sectie Aigeiros. In hoeverre nog andere vormen van Marssonina voorkomen in de secties Leuce en Tacamahaca zal een onderwerp van voortgezet onderzoek zijn.*

De inoculaties werden uitgevoerd op bladschijfjes, die in petrischalen op water drijven. Deze werkwijze heeft als voornaamste voordeel dat men onder gecontroleerde omstandigheden kan werken, maar als nadeel dat men met bladstukken werkt en dat deze bovendien onder omstandigheden verkeren, die niet vergelijkbaar zijn met de natuurlijke. Dit maakt het noodzakelijk de hier verkregen resultaten te vergelijken met die van de inoculaties op levende planten. Gebleken is dat de levensduur van de bladschijfjes zeer uiteenlopend is. Met het verstrijken van het groeiseizoen van de planten buiten neemt ook de levensduur van de bladschijfjes af. Door toevoeging van kaliumnitraat

*) Verschijnt tevens als Korte Mededeling 64 van het Bosbouwproefstation.

en dextrose aan het water in de petri-schalen werd de aantasting door Marssonina in belangrijke mate gereduceerd (serie VII).

De aantasting door Marssonina wordt dus blijkbaar niet uitsluitend bepaald door de aanwezigheid van inoculum, respectievelijk ascosporen en conidiën, maar mede door de chemisch-fysiologische toestand, waarin het blad verkeert. Naar analogie van een onderzoek verricht bij de appel (Mann & Wallace) wordt de veronderstelling geuit dat ook hier aan regen en wind een uitlogend effect moeten worden toegeschreven, waardoor de kali uit het blad verdwijnt. Dit heeft invloed op de fotosynthese en doet de resistentie tegen Marssonina afnemen.

Daar juist die beplantingen, die het meest geëxponeerd zijn, het hevigst van deze ziekte lijden, terwijl direct aangrenzende bomen, op beschutte plaatsen, weinig of niet zijn aangetast, wordt aan de invloed van de kali gedacht. Er zijn namelijk aanwijzingen dat kalibemestingen op kali-arme gronden een gunstig effect hebben op de mate van aantasting. Het een en ander wordt echter sterk beïnvloed door plaatselijke omstandigheden, waarbij in de polders wellicht het kalium-calcium antagonisme van de grond een woordje meespreekt.

Since 1960 a serious leaf spot disease of poplars caused by species of *Marssonina* has occurred in various parts of the Netherlands, especially in the new polders, forming an important threat for poplar growing. Attack by this leaf spot fungus is particularly serious along the roads, but it may also prevail in stands. Various clones of the section *Aigeiros* Duby and species op poplars belonging to the sections *Leuce* Duby and *Tacamahaca* Spach are affected. On the leaves two types of leaf spot may be recognized, one with brown, necrotic dots about 1 mm in diameter, irregularly spread on the whole leaf surface and sometimes on the petiole and the other with tan or olive-brown, more or less roundish spots, about 4 to 5 mm in diameter. The dots as well as the spots gradually increase in numbers, and as a result the leaves are dropped. Early defoliation of the trees may give considerable decrease in growth and consequently timber production. A repeated early defoliation during a number of subsequent years is followed by a physiological disturbance of tree growth.

The life history of this pathogen is characterized by two reproductive stages. During the growing season a parasitic stage occurs on the green leaves developing macroconidial acervuli, followed in the season by microconidial acervuli. The acervuli produced on the dots contain macroconidia 9 - 17 $\times$ 4 - 7 μ; the acervuli developed on the spots contain conidia 17 - 24 $\times$ 6 - 11 μ. Characteristic haustoria were observed in the host tissue. In the autumn initials of the apothecial form are found on the dead fallen leaves. These apothecia mature in the spring and discharge their ascospores which cause primary infection of the new leaves (Gremmen, 1962).

This short account is the result of a series of experiments dealing with the parasitic behaviour of Marssonina on discs of poplar leaves. This leaf disc method formerly used by Van Vloten (1944) for a study of poplar rust has been found to be extremely useful for testing various species or clones of poplars for their susceptibility to the rust fungus.

The discs used in these experiments were punched from healthy, mature leaves and afterwards carefully floated on tap water in clean petri-dishes.

The great advantage of this method is the experimental work done under controlled conditions, which avoids secondary infection.

On the other hand these discs cannot be compared with living leaves and consequently the work described here may only give some preliminary information which ought to be completed with life history studies on the living tree itself.

Inoculations with Ascospores

Leaves of cv Marilandica from Heerde and leaves from *Populus alba* from Wageningen both affected by the Marssonina leaf spot fungus were collected in the autumn and overwintered in flower pots.

In the spring, small leaf fragments bearing mature apothecia of the *Drepanopeziza* stage, were stuck to the inside of a number of petri-dish covers, in which leaf discs were floating on tap water. The upper- as well as the underside of the leaf discs were exposed to ascospore discharge (series I and II).

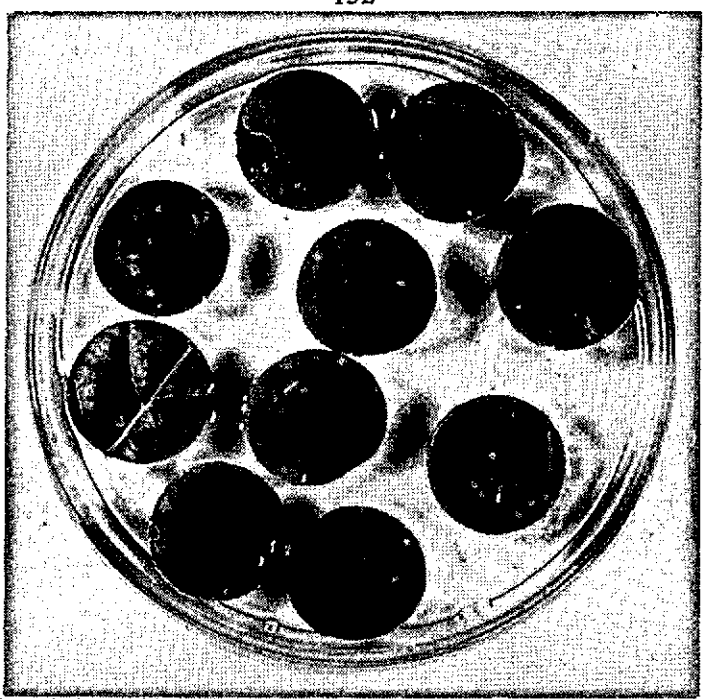
Series I and II		Percentage infected leaf discs*) *Percentage geïnfecteerde bladschijfjes*			
Hosts *Waardplanten*		Ascospores derived from *Ascosporen afkomstig van*			
		cv Marilandica (Aigeiros), (Heerde)		Pop. alba (Leuce) (Wageningen)	
		series I	II	I	II
Populus alba (Leuce)	Upperside *Bovenkant*	0%	0%	100%	44%
Populus alba	Underside *Onderkant*	0%	0%	100%	44%
cv Heidemij (Aigeiros)	Upperside *Bovenkant*	47%	33%	0%	0%
cv Heidemij	Underside *Onderkant*	33%	44%	0%	0%

* All checks remained free from infections

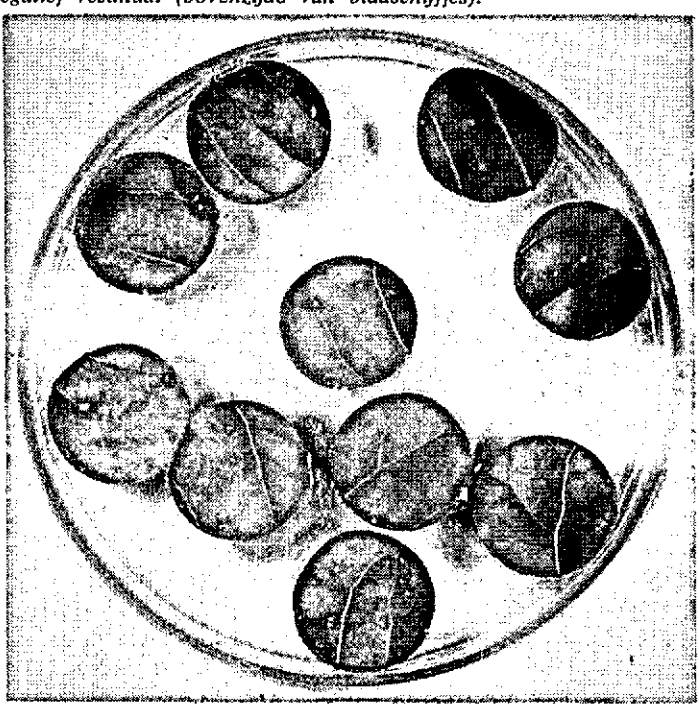
Ascospores from apothecia from leaves of cv Marilandica (Aigeiros) infected Heidemij, a clone of the same section, but did not infect *Populus alba*. Ascospores from *Populus alba* only reinfected *Populus alba* but not cv Heidemij. It has been demonstrated too that both sides of the leaves may be infected. In both cases the minute type of dots have been developed. In the cv Heidemij infection has been noticed after a ten day's period; in *Populus alba* after about twenty days.

Inoculations with conidia

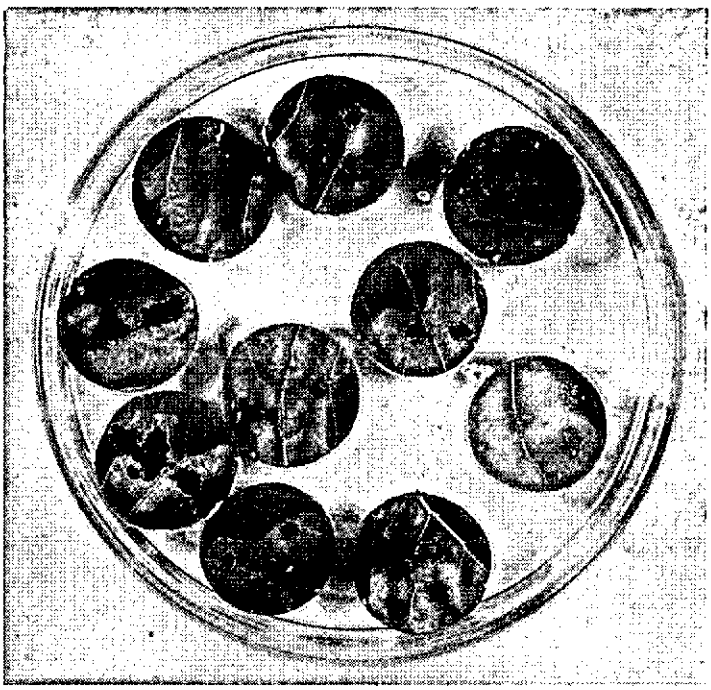
In the next experiment (III) leaf discs were inoculated with conidia obtained from cv Heidemij and with conidia from *Populus alba* both from series I. It has been demonstrated again that conidia derived from Aigeiros material are able to infect related cultivars and moreover *Populus candicans*. Conidia from *Populus alba* did not infect *Populus alba* Var. *nivea*, neither clones of the section Aigeiros, or *Populus candicans*.



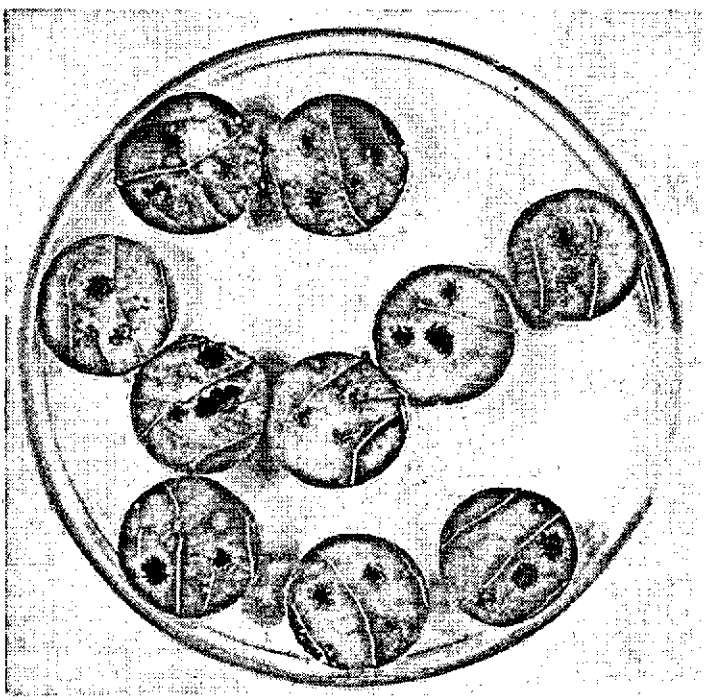
Afb. 1. Inoculations with conidia of „alba" strain on cv Regenerata (Aigeiros) showing negative results on upperside of leaf discs.
Inoculaties met conidiën van „alba" ras op de cv Regenerata (Aigeiros) met negatief resultaat (bovenzijde van bladschijfjes).



Afb. 2. Inoculations with conidia of „alba" strain on cv. Regenerata (Aigeiros) showing negative results on underside of leaf discs.
Inoculaties met conidiën van „alba" ras op de cv Regenerata (Aigeiros) met negatief resultaat (onderzijde van bladschijfjes).



Afb. 3. Inoculations with conidia of „Aigeiros" strain on cv Regenerata (Aigeiros) showing positive results on upperside of leafdiscs.
Inoculaties met conidiën van „Aigeiros" ras op de cv. Regenerata (Aigeiros) met positief resultaat (bovenzijde van bladschijfjes).



Afb. 4. Inoculations with conidia of „Aigeiros" strain on cv. Regenerata (Aigeiros) showing positive results on underside of leaf discs.
Inoculaties met conidiën van „Aigeiros" ras op de cv Regenerata (Aigeiros) met positief resultaat (onderzijde van bladschijfjes).

Series III		Conidia derived from*) *Conidiën afkomstig van*			
Hostplants *Waardplanten*		cv Heidemij (Aigeiros)		Populus alba (Leuce)	
		Upperside *Bovenkant*	Underside *Onderkant*	Upperside *Bovenkant*	Underside *Onderkant*
cv Serotina	(Aigeiros)	+	+	1 ac. **)	1 ac. **)
cv Marilandica	(Aigeiros)	+	+	—	—
cv Robusta	(Aigeiros)	+	+	—	2 ac. **)
cv Gelrica	(Aigeiros)	+	+	1 ac. **)	—
cv Regenerata	(Aigeiros)	+	+	—	2 ac. **)
Populus candicans (Tacamahaca)		+	+	—	—
Populus alba var. nivea (Leuce)		—	—	—	—

*)all checks were negative
**) spontaneous infections of the leaves from abroad.

In the next experiment cv Marilandica leaves with characteristic dots were collected in the nursery. After they have been shaken in tap water a spore suspension was obtained containing 15 à 20 thousand conidia per cm^3. The upper- as well as the underside of a series of leaf discs has been inoculated with this suspension (series IV).

Serie IV		Percentage infected leaf discs*) Percentage geïnfecteerde bladschijfjes		
Hostplants *Waardplanten*		Conidia from cv Marilandica *Conidiën van cv Marilandica*		
		Upperside *Bovenkant*	Underside *Onderkant*	Check *Control*
cv Serotina	(Aigeiros)	63%	100%	0%
cv Heidemij	(Aigeiros)	100%	63%	0%
cv Regenerata	(Aigeiros)	75%	75%	0%
cv I 214	(Aigeiros)	63%	87%	0%
cv Marilandica	(Aigeiros)	50%	75%	0%
cv Robusta	(Aigeiros)	38%	75%	0%
cv Gelrica	(Aigeiros)	38%	75%	0%
NL 925	(Aigeiros)	50%	50%	0%

*) All checks free from infections

The clones used in this experiment have been arranged in sequence of attack: cv Serotina and cv Heidemij showing the heaviest attack; NL 925 the lowest.

Series V has been devised to compare "dots" and "spots" on leaf discs. For this purpose a number of discs were inoculated with conidia of cv Marilandica from Heerde taken from series III, representing dots; another series of discs were inoculated with conidia of cv Gelrica, representing spots. Leaves of the latter clone attacked by Marssonina had been collected in the nursery at Wageningen and afterwards shaken in tap water. Both suspensions were used to inoculate a series of clones viz. Serotina, Heidemij, Regenerata, I 214, Marilandica, Robusta, Gelrica and NL 925.

In the first category the discs again developed characteristic minute dots with acervuli containing 13 - 18 × 5 - 9 μ; in the second group however, only a few spots developed, but contrary to expectation numerous dots. Obviously the spore suspension used for inoculation of the second consisted of a mixture of both spores, presumably the leaves of cv Gelrica must have been colonised by both types of fungi.

Series VI has been projected in the same way as series V. Conidia of dots from cv Regenerata from series III and conidia of spots taken from fresh leaves of crossing 1048 × 1038 were used for inoculating discs of the cv Gelrica. The spore suspension from the spots was carefully prepared by checking each leaf under the microscope in order to avoid infection with the other type of conidia. Two similar groups of discs were inoculated and the results after a five weeks period were as follows: 35,7% of the total number of inoculations with the small conidia produced dots with their characteristic conidia and 36,2% of the inoculations with the other type of conidia produced spots with its own spore type. From this experiment it is obvious that each type of spot is connected with a characteristic spore form pointing out a difference in fungus species.

A great disadvantage of this leaf disc method, however, is the fact that soluble substances from the discs are diffusing into the surrounding water. The longevity of the discs seems to be determined by the cell contents and depends on the time of the year when they are picked from the trees; the age of the leaf and its situation on the branch. Doubtless this phenomenon, the dialysis of cell soluble substances must also have important consequences as to parasitism of Marssonina.

In the next experiment (VII) half of the discs were floating on tap water; the other half on tap water with 1 gram of potassium nitrate and 20 grams dextrose per liter. Inoculations have been performed on cv Gelrica and cv Robusta, using conidia from dots from cv Regenerata (series V), and conidia from spots collected from fresh leaves of the crossing 1034 × 1035. The results after a three weeks period are given below.

Series VII		Percentage of infections *Percentage infecties*	
Hosts *Waardplanten*		Conidia derived from *Conidiën afkomstig van*	
		cv Regenerata (Aigeiros) (dots)	1034 x 1035 (spots)
cv Gelrica			
	on tap water	13%	48%
	on nutrient solution	15%	10%
cv Robusta			
	on tap water	10%	20%
	on nutrient solution	3%	5%

All checks 0%.

This experiment indicates that an addition of certain nutrient substances to tap water has a tendency to decrease the percentage of infection caused by Marssonina on leaf discs.

Discussion of the results

These preliminary results concerning the development of Marssonina on leaf discs demonstrate that inoculations with ascospores as well as conidia from "dots" derived from Aigeiros poplars may infect members of the same section and *Populus candicans* Ait. (section *Tacamahaca* Spach), but not *Populus alba* L. (section *Leuce* Duby). Ascospores and conidia from Marssonina species inhabiting *Populus alba* may only infect *Populus alba*, but will not infect clones of the section *Aigeiros* Duby (cf. series I, II and III).

Ascospores as well as conidia are capable of infecting both sides of the leaf discs (series III, IV). Corke (1954), working with the black currant leaf spot caused by *Pseudopeziza ribis* Kleb. a relative of Marssonina, found after inoculation of both surfaces of leaves with ascospores and conidia that these were only successful on the lower surfaces.

By inoculating a series of Aigeiros poplars with conidia of dots some information has been obtained as to their susceptibility. The clones mentioned in series IV have been arranged in sequence of susceptibility: Serotina being most susceptible and NL 925 most resistant.

Inoculations with conidia from dots always reproduce dots with the smaller type of conidia (9 - 17 $\times$ 4 - 7 μ); inoculations with conidia from spots always develop spots with the other spore type (17 - 24 $\times$ 6 - 11 μ) (cf. series VI).

During the course of the work it has been noticed that the longevity of the discs on the water medium may vary considerably. To improve their duration on this medium certain nutrient substances have been added. A striking influence has been observed when comparing fungus pathogenicity on discs floating on tap water to those floating on water with potassium nitrate and dextrose. Whether this is caused by the potassium salt or the sugar or combination of both must be studied further (series VII).

In this connection it is of interest to refer to the work of Mann & Wallace (1925). These authors demonstrate that necrotic spots on apple leaves, the result of abnormal wet weather conditions in certain years, are caused by a leaching effect of rain and wind influencing the potassium contents of these leaves. When they immersed healthy apple leaves in cold water for certain periods two processes were involved viz. (1) a penetration of the water into the intercellular spaces and (2) dialysis of soluble compounds from the cells into the water. Their experiments demonstrate an important diffusion of the potassium salts into the water and they suggest that under field conditions the same phenomenon takes place when leaves are constantly washed by rain in combination with strong winds.

Since poplars most affected in our polders are to be found along the roads, where these trees are heavily exposed to strong winds and rain, the same clones standing on sheltered places e.g. on nearby farm yards, show a lower degree of attack.

Many indications concerning the significance of potassium for poplars have been observed. Fertilization with wood-ash, giving an increment of the potassium in the leaf from 0,6 to 2%, considerably lowered attack by Marssonina (Van der Meiden, 1962). In a report on the occurrence of Marssonina over the year 1961 (Anonymus, 1962) some final conclusions have been made; (1) Trees with slight potassium deficiency are slightly attacked by Marssonina; (2) Trees with potassium deficiency show a heavier attack by Marssonina than trees without potassium deficiency, and (3) heavy potassium fertilization influence the degree of attack in a favourable sense.

Summarizing we may conclude that infection by Marssonina depends at least on two main factors. (1) The availability of "air-borne" inoculum transported by prevailing winds from certain localities and filtered by road plantations and (2) an increased possibility of infection by interference of the potassium balance in the leaves, probably caused by a leaching effect of wind and rain.

It is well known that potassium deficiency general lowers plant resistance against parasites since it influences photosynthesis. Beside these major factors, some minor local agencies may be of influence too e.g. in the polders the so called Calcium Potassium antagonism may also play an important role.

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