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Screening Lactuca for resistance to Myzus persicae

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Abstract

A macrotest was developed to screen rapidly many genotypes of *Lactuca* in the greenhouse for resistance to *Myzus persicae*. In this test the estimated number of aphids on certain leaves of plants artificially exposed to aphid infestation was representative for the total number of aphids per plant. Significant differences in resistance between genotypes were observed and several partially resistant PIVT numbers were found among 645 accessions tested in 1973 and 1974.

Also a microtest with leaf cages was developed in which biomass after seven to eight days of larval development and net larvae production during four to seven days functioned as criteria of resistance. Besides differences between genotypes, also within plants and between plants of the same genotype significant differences were found to occur. After testing nineteen genotypes with different levels of resistance it appeared that there was a clear relationship between results from both types of tests.

Introduction

The green peach aphid, *Myzus persicae* (Sulzer), causes problems in glasshouse lettuce (*Lactuca sativa* L.) growing and has to be controlled with insecticides. Safer methods of insect population control are desirable to prevent adverse effects of the insecticides. In this respect resistant varieties could be of great help (Van Marrewijk and De Ponti, 1975), but such varieties are not yet available. Therefore the following research programme was initiated:

1. Development of tests for resistance and search for resistant progenitors.
2. Research on the genetics and the morphological, physiological and/or biochemical backgrounds of the resistance.
3. Transfer of resistance to cultivated types.

Results of research mentioned under 1) are presented in this paper.

Materials and methods

Macrotest. A macrotest was developed for rapid screening of many genotypes for resistance to *Myzus persicae*. For this purpose PIVT (Plant Introduction IVT) numbers were placed in a glasshouse in four randomized blocks with 5 to 6 plants per number per block. About five weeks after sowing, when the plants had 5 to 8 leaves of medium size, they were exposed to aphid infestation.

Aphids were reared on Chinese cabbage, *Brassica cernua* (Thbg.) Forbes and Hemsl cv. Granaat, under long-day conditions and at day and night temperatures of about

Fig. 1. Leaves of different size and shape with dots representing aphids.

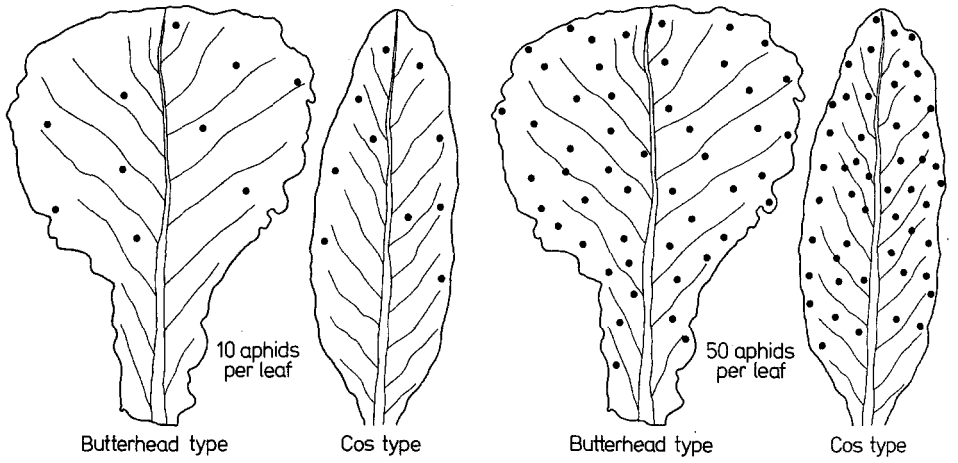

Fig. 1. Bladeren van verschillende afmeting en vorm met stippen die bladluizen voorstellen.

20 and 16 °C, respectively. Chinese cabbage leaves with aphids were detached and placed at 14 °C half a day before transfer. Then the aphids were shaken off above plastic trays containing moist filter paper and about 500 lettuce leaf discs (diam. 2 cm). They settled rapidly on the lower surface of the leaf discs. One disc with 10 to 15 aphids (larvae and adults), was placed on each plant to be tested whereafter aphid populations developed. As the aphids were mostly rather evenly distributed over the lower side of the older leaves of a plant, estimation of the number of aphids on one of these leaves was considered representative. For estimating, the plants were cut off and turned upside down. The leaves with aphids were then compared with drawings of leaves of varying size and shape with distributions of dots representing aphids (scale 0, 10, 20, ..., 90) (Fig. 1). Different sizes and shapes of drawings were necessary for a reliable estimate per leaf. Each replicate was evaluated by one person.

Microtest. For our future research on the genetics of resistance to *M. persicae* individual plants will have to be tested. For such tests, leaf cages (diam. 2 cm, height 0.8 cm) were used (Fig. 2) which were placed on plants grown in 10 l plastic pots with Trio potting soil.

In each cage ten larvae, less than 24 h old, were enclosed. After seven days the surviving, nearly adult aphids were counted (survival) and weighed (biomass). From these, five apterous aphids were chosen at random and returned to the cages. The following eight days larvae production per cage was determined, the young larvae were removed daily and gross and net larvae production calculated. Gross production means the sum over seven days of the average daily larvae production per adult and net production means the ratio of the total larvae production over the original number of adults (usually five). Gross and net production were compared to investigate the influence of mortality of adults on larvae production with different genotypes. Unless stated otherwise, experiments were carried out at natural light conditions and temperatures varying between 20 °C (day) and 16 °C (night). For

Fig. 2. Lettuce plant with leaf cages.

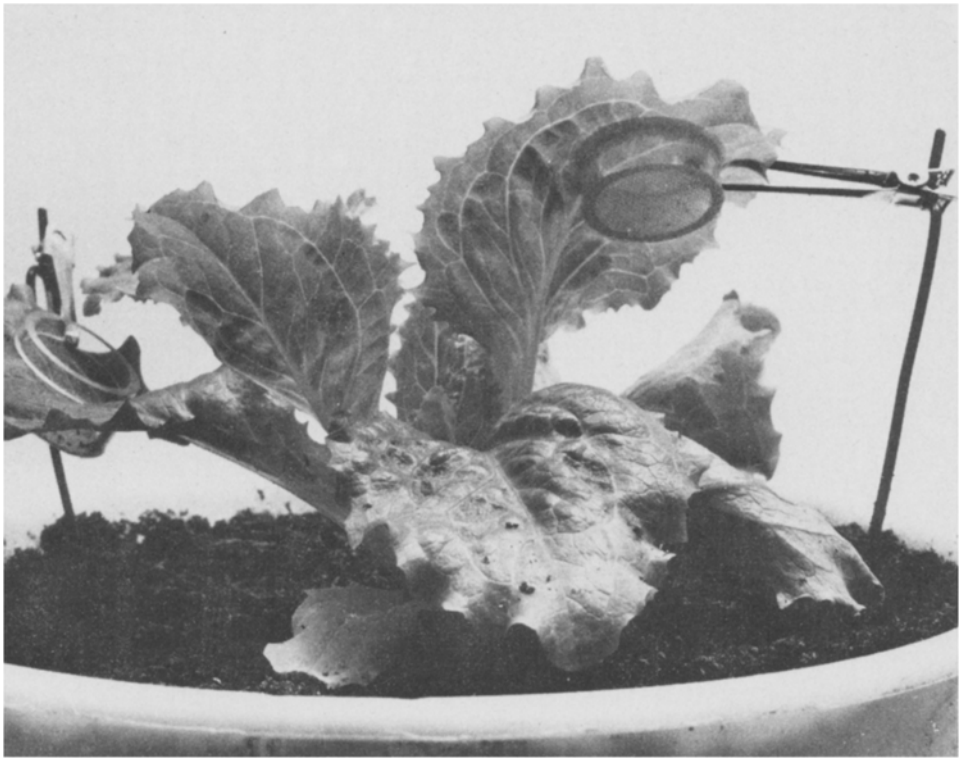

Fig. 2. Slaplant met bladkooitjes.

certain experiments use was made of artificial illumination of 16 h daylength from HPIT lamps (400 Watt) with one lamp per 2 m^2 at 1.5 m above the plants. Further details of materials and methods are given under 'Results'.

Results and discussion

Search for resistance by macrotest. In a glasshouse at a temperature varying between 25 °C (day) and 15 °C (night), 645 PIVT numbers of the IVT *Lactuca* gene bank were screened with the macrotest for resistance in August 1973 or May 1974. Aphid numbers were estimated on one (1973) and three plants (1973, 1974) per PIVT number per replicate, two and five weeks after exposure to aphids, respectively.

Estimates two weeks after aphid introduction did not reveal significant differences between the various PIVT numbers tested. The results of analyses of variance for estimates five weeks after aphid introduction are shown in Table 1. It appears that in both years very significant differences occurred between genotypes for the estimated number of aphids per leaf. In 1973 this number varied between 14 (PIVT 42) and 74 (PIVT 312) and in 1974 between 10 (PIVT 504) and 83 (PIVT 572).

It is unlikely that these differences resulted from non-preference (De Ponti et al., 1975) because between plants practically no migration was observed. Therefore they

Table 1. Analyses of variance for the estimated numbers of aphids per leaf five weeks after exposure to aphid infestation for 372 (1973) and 273 (1974) PIVT numbers.

Source	1973 test			1974 test		
	D.F.	M.S.	F	D.F.	M.S.	F
PIVT numbers	371	496	4.5**	272	747	3.3**
Replicates	3	22668	206.5**	3	1942	8.6**
Rest	1113	110		853	227	
Mean		37.2			34.3	
Standard deviation		10.5			15.1	

** Significant at 1% level.

Tabel 1. Variantie-analyses voor de geschatte aantallen bladluizen per blad, vijf weken na blootstelling aan bladluisaantasting bij 372 (1973) en 273 (1974) PIVT-numbers.

are ascribed to differences in resistance. In both years significant differences also occurred between replicates.

Comparison of estimates and counts in the macrotest. To investigate the reliability of estimating the aphid numbers, a comparison was made with direct counting. An experiment was carried out in May 1975 with five susceptible and fourteen partially resistant PIVT-numbers, as appeared from the 1973 and 1974 experiments.

In each of two glasshouse compartments genotypes were tested in four replicates with six plants per genotype per replicate. In one compartment the plants were exposed to aphids when they had five to eight rather large leaves (exposure I). In the other compartment aphids were introduced two weeks later when plants were almost fully grown (exposure II). The aphids on one plant per PIVT number per replicate were counted after two and four weeks of exposure I while the number of aphids per leaf was estimated on one and three other plants, respectively. For exposure II, the aphids on one plant per number per replicate were counted after two and four weeks, and after four weeks the number per leaf was estimated on the remaining plants.

Results of the analyses of variance of the figures obtained from counting and estimating are shown in Table 2. For all counts and estimates with exposures I and II significant differences were found between the genotypes investigated. Hence, the occurrence of these differences is neither influenced by time and method of evaluation nor by plant age.

The means for counts of exposure II, especially with the susceptible genotypes were greater than those for exposure I. This indicates that population development is faster on old than on young plants. Per exposure, means for the first count were smaller than those of the second count. However, the means for numbers of aphids from the first and second estimate with exposure I and also those from the last estimates of exposures I and II practically did not differ. In view of the above mentioned differences between the various counts this implies that by estimating only relative differences are observed. From Fig. 3, 4 and 5 in which the relationship between counts and estimates of the number of aphids per genotype is shown, it appears that

Table 2. Analyses of variance for the counted (in hundreds) numbers of aphids per plant and the estimated numbers of aphids per leaf with 19 PIVT numbers.

Source	D.F.	Exposure I								Exposure II					
		counts after				estimates after				counts after				estimates after	
		2 weeks		4 weeks		2 weeks		4 weeks		2 weeks		4 weeks		4 weeks	
		M.S.	F	M.S.	F	M.S.	F	M.S.	F	M.S.	F	M.S.	F	M.S.	F
Genotypes	18	68.3	11.6**	386.9	13.7**	941	8.4**	1219	12.2**	181.2	4.8**	1308.4	7.8**	1423	15.4**
Replicates	3	27.1	4.6**	40.4	1.4	1112	10.0**	663	6.6**	122.1	3.3*	922.1	5.5**	1155	12.5**
Rest	54	5.9		28.3		111		99		37.4		167.1		92	
Mean		7.5		16.1		52		55		9.4		24.5		52	
Standard deviation		2.4		5.3		11		10		6.1		12.9		10	

* Significant at 5% level; ** Significant at 1% level.

Tabel 2. Variantie-analyses voor de getelde (in eenheden van honderd) aantallen bladluizen per plant en de geschatte aantallen bladluizen per blad bij 19 PIVT-nummers.

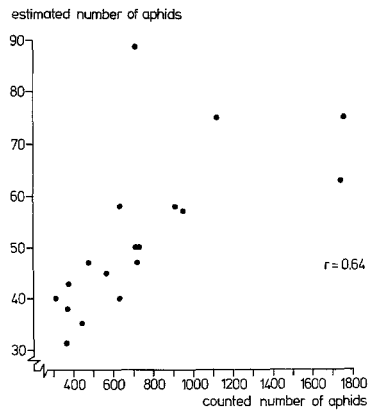

Fig. 3. Relationship between means of counted number of aphids per plant and estimated number of aphids per leaf for eighteen genotypes two weeks after exposure I.

Fig. 3. Verband tussen gemiddelden van getelde aantallen bladluizen per plant en geschatte aantallen bladluizen per blad voor achttien genotypen twee weken na blootstelling I.

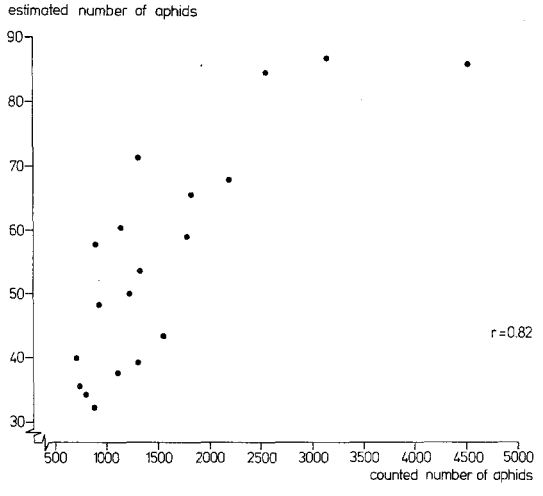

Fig. 4. Relationship between means of counted number of aphids per plant and estimated number of aphids per leaf for nineteen genotypes four weeks after exposure I.

Fig. 4. Verband tussen gemiddelden van getelde aantallen bladluizen per plant en geschatte aantallen bladluizen per blad voor negentien genotypen, vier weken na blootstelling I.

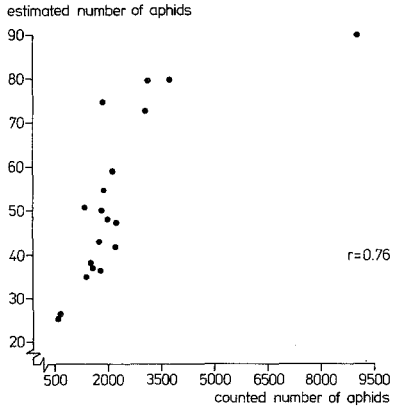

Fig. 5. Relationship between means of counted number of aphids per plant and estimated number of aphids per leaf for nineteen genotypes four weeks after exposure II.

Fig. 5. Verband tussen gemiddelden voor getelde aantallen bladluizen per plant en geschatte aantallen bladluizen per blad voor negentien genotypen, vier weken na blootstelling II.

they agree well. This indicates that macrotests, estimating the number of aphids per leaf are useful in screening for resistance.

Microtest and variation within and between plants. Variation within plants and between plants of the same genotype for biomass and larvae production was investigated with the microtest in a glasshouse in March and April 1975 with two groups of ten plants each (exposure I and II) of the susceptible genotype PIVT 197 and of the partially resistant genotype PIVT 180. There was an interval of seven days between exposures I and II.

Cages were placed on the lower surface of an older and of a rather young leaf of each plant, two cages at the base, two at the middel and two at the top of the leaf, each time on both sides of the main vein. Ten larvae were enclosed in each cage. Then, biomass and larvae production were determined and analyses of variance were carried out. Results are shown in Tables 3 to 6. As the results for gross and net production agreed very well, only the figures for the latter are presented.

Table 3 shows that with exposures I and II within plants between the leaf cage positions (leaf age and/or part) significant differences for biomass occurred with the partially resistant genotype and also with exposure II of the susceptible genotype. Significant differences also occurred between plants.

The mean biomass for different leaf ages and leaf parts with the partially resistant and susceptible genotype are presented in Table 4. According to the range test of Keuls (1952) with the susceptible genotype only with exposure II on old leaves significantly more biomass was produced than on young leaves. With the partially resistant genotype biomass production on old leaves near the leaf tops was significantly greater than at other cage positions.

Table 3. Analyses of variance for biomass (in 10 μg) after seven days of larval development on two PIVT numbers.

Source	PIVT 197 (susceptible) exposure				PIVT 180 (partially resistant) exposure			
	I		II		I		II	
	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Between plants (P)	9	62.0	9	79.5*	9	140.1**	9	69.6
Leaf age (La)	1	133.8	1	719.7**	1	276.1**	1	180.0*
(P) × (La)	9	53.7*	9	7.9	9	14.6	9	32.9
Leaf part (Lp)			2	1.4	2	93.2*		
(Lp) × (La)			2	11.1	2	46.7		
(Lp) × {(Lp) × (La)}	3	65.3					3	105.9*
Rest	26	23.1	35	34.6	36	21.8	27	26.4
Mean		40.0		39.1		22.6		20.5
Standard deviation		4.8		5.4		4.5		5.1

* Significant at 5% level; ** Significant at 1% level.

Tabel 3. Variantie-analyses voor biomassa (in 10 µg) na zeven dagen larvale ontwikkeling op twee PIVT-nummers.

Table 4. Means and range for biomass (in μg) after larval development on different leaves and leaf parts with two PIVT numbers.

Leaf		PIVT 197 (susceptible)				PIVT 180 (partially resistant)			
age	part	exposure I		exposure II		exposure I		exposure II	
		mean	range	mean	range	mean	range	mean	range
Old	basis			431	200	206	135		
	middle	423	112	425	125	264	195	192	184
	top	416	200	421	166	274	257	265	243
	mean	420		426		247		229	
Young	basis	421	208	347	201	198	324	195	154
	middle	378	238	356	168	252	78	171	135
	top	360	155	367	148	214	145	203	230
	mean	386		357		204		190	
Overall	mean	400		391		226		205	

Tabel 4. Gemiddelden en spreiding voor biomassa (in μg) na een larvale ontwikkeling op verschillende bladeren en bladdelen bij twee PIVT-nummers.

The results of the analyses of variance for net larvae production in Table 5 show that in this respect no significant differences occurred within and between plants with the susceptible genotype. Differences within plants did occur with the partially resistant genotype, both with exposures I and II.

Table 5. Analyses of variance for net larvae production per adult per day (means (n) of seven days) after $\sqrt{n+1}$ transformation then multiplied by 10, with two PIVT numbers.

Source	PIVT 197 (susceptible) exposure				PIVT 180 (partially resistant) exposure			
	I		II		I		II	
	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Between plants (P)	9	2.9	9	3.7	9	5.1	9	3.7
Leaf age (La)	1	0.4	1	0.3	1	3.8	1	1.4
(P) × (La)	9	2.9	9	1.6	9	1.7	9	1.4
Leaf part (Lp)			2	1.2				
(Lp) × (La)			2	0.6				
(Lp) + {(Lp) × (La)}	3	0.3			3	6.0**	3	10.4**
Rest	26	2.3	35	2.3	27	1.2	27	1.7
Mean		20.2		20.3		13.5		12.9
Standard deviation		1.5		1.5		1.1		1.3

** Significant at 1% level.

Tabel 5. Variantie-analyses voor netto larvenproduktie per adult per dag (gemiddelden (n) van zeven dagen) na $\sqrt{n+1}$ transformatie en vermenigvuldigd met 10, bij twee PIVT-nummers.

Table 6. Means and range for net larvae production in seven days on different leaves and leaf parts of two PIVT numbers.

Leaf		PIVT 197 (susceptible)				PIVT 180 (partially resistant)			
age	part	exposure I		exposure II		exposure I		exposure II	
		mean	range	mean	range	mean	range	mean	range
Old	basis			21.8	16.3				
	middle	21.0	10.5	22.9	8.7	5.7	7.6	2.7	6.1
	top	21.8	14.2	21.3	8.7	6.7	10.3	5.9	8.8
	mean	21.5		22.1		6.3		4.3	
Young	basis	21.6	12.8	20.4	10.0	3.4	8.7	3.2	7.1
	middle	21.6	14.3	22.2	12.8	5.9	4.9	5.0	7.6
	top	22.4	9.1	21.8	13.7	6.5	10.8	6.3	9.9
	mean	21.8		21.6		4.6		4.8	
Overall mean		21.7		21.9		5.5		4.6	

Tabel 6. Gemiddelden en spreiding voor netto larvenproduktie in zeven dagen op verschillende bladeren en bladdelen van twee PIVT-nummers.

Table 6 shows the means for net larvae production per leaf age and per leaf part. According to the range test of Keuls, with exposures I and II of the partially resistant plants, near the base of young leaves significantly less larvae were produced than on other leaf parts.

From the figures for variation between plants for biomass (Table 4) and larvae production (Table 6) it appears that rather often variation was smalles if the cages were situated on the middle of the lower leaf surface.

Significant differences within plants and between plants of the same genotype for biomass or net larvae production may result from morphological, anatomical and/or biochemical differences. Part of the differences in the above results, however, may result from differences between aphids for parasitic ability. For in the tests, per group of cages (e.g. per leaf part) often one larvae progeny or a composite of a small number of progenies was used. Consequently the larvae populations on different leaves or leaf parts may have differed for parasitic ability because in spite of parthenogenetic larvae production genetic differences for this ability may occur between progenies. These differences will more likely be expressed on (partially) resistant genotypes than on susceptible ones.

Significant differences between plants of the same genotype for biomass or larvae production will cause problems in testing genotypes for resistance. These problems may partly be overcome by using a certain number of plants of each genotype to be tested. Based on a coefficient of variation of 30% for the mean of two cages per position on the leaf an approximation is given in Table 7 of the numbers of plants per genotype, necessary for detection of certain differences in resistance. This table has been derived from the nomograph for the power of the F-test by Ferguson (1962). These estimates might also be used for testing individual plants of segregating popu-

Table 7. The minimum number of plants per genotype to be tested for distinguishing different levels of resistance.

Number of plants per genotype necessary for distinction	Resistance differences (in %) to be distinguished	
	with 70% probability	with 50% probability
5	60	50
6	54	45
8	45	37
10	39	33
12	36	30
14	33	28
16	30	26
18	29	24
20	27	23

Tabel 7. Het minimum aantal planten per genotype dat getoetst moet worden om verschillende resistentieniveaus te kunnen onderscheiden.

lations. The number of plants indicated in Table 7 should then represent the number of pairs of leaf cages at the middle of the lower surface of similar leaves of one plant.

Comparison of macro- and microtests. The usefulness of the microtest was investigated by comparing with the macrotest. For this purpose plants of the 19 genotypes mentioned earlier were placed in a glasshouse in September-October 1975 with artificial illumination and usual temperature conditions.

Four replicates with five plants per genotype per replicate were used for the macrotest. For the microtest two replicates with four plants per genotype per replicate were used with two leaf cages per plant one on each side of the midrib on the middle of the lower surface of a moderately old leaf. The number of aphids was estimated in the macrotest while survival percentage, biomass and net larvae production for the first three, four, five, six, seven and eight production days were determined for the microtest. This last was done to find out after which production period differences between genotypes were maximal.

The results of analyses of variance for the number of estimated aphids (macrotest), survival, biomass, and net larvae production (microtest) are shown in Table 8. For net production only the results of the first four production days are mentioned because then differences between genotypes were most significant.

A great variation was found for estimates of the number of aphids per leaf, biomass and net larvae production and for these characters very significant differences occurred between genotypes. Fig. 6–8, showing the relationships between the above characters, indicate both the correspondence between macro- en microtest and between biomass and net larvae production.

The above means that both test methods can be used for investigating the level of resistance. However, because of the variation between plants of the same genotype in microtests, these tests should be further improved for research on the inheritance of (partial) resistance.

Table 8. Analyses of variance for four characters which may function as selection criteria for resistance.

Source	Estimates of number of aphids per leaf			Percentage of survivals			Biomass (in 10 μg) after seven days			Net production in four days		
	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F	D.F.	M.S.	F
Genotypes	18	2049	10.7**	18	67	0.6	18	72.9	3.7	18	10.2	7.3**
Replicates	3	929	4.9**	1	342	3.2	1	271.4	13.8**	1	3.2	2.3
Rest	54	191		18	108		18	19.7		18	1.4	
Mean		52.0			62.2			23.7			3.6	
Standard deviation		13.8			10.4			4.4			1.2	

** significant at 1 % level

Tabel 8. Variantie-analyses voor vier eigenschappen die misschien kunnen fungeren als selectiecriteria voor resistentie.

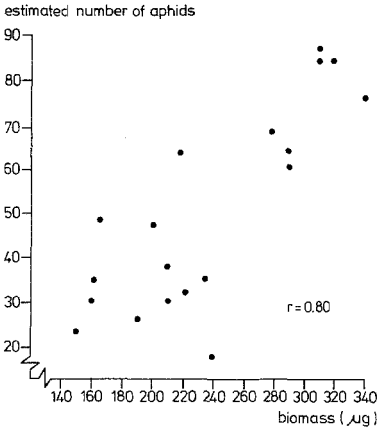

Fig. 6. Relationship between means of biomass (in μg) after seven days of larval development and estimated number of aphids per leaf for nineteen genotypes.

Fig. 6. Verband tussen gemiddelden voor biomassa (in μg) na zeven dagen larvale ontwikkeling en het geschatte aantal bladluizen per blad voor negentien genotypen.

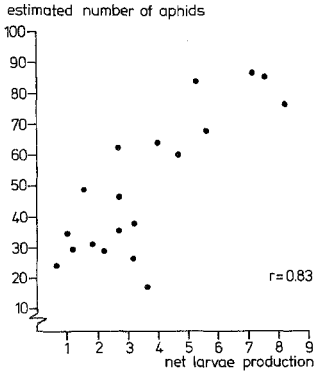

Fig. 7. Relationship between means of net larvae production in four days and estimated number of aphids per leaf for nineteen genotypes.

Fig. 7. Verband tussen gemiddelden voor netto larvenproduktie in vier dagen en het geschatte aantal bladluizen per blad voor negentien genotypen.

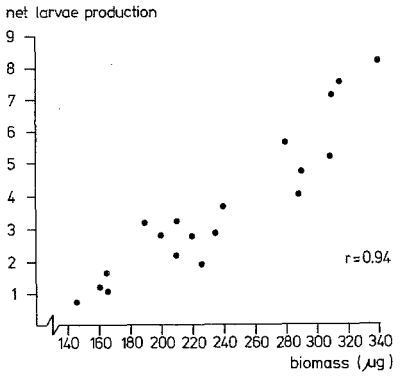

Fig. 8. Relationship between means of biomass (in µg) after seven days of larval development and net larvae production in four days for nineteen genotypes.

Fig. 8. Verband tussen gemiddelden voor biomassa (in µg) na zeven dagen larvale ontwikkeling en netto larvenproduktie in vier dagen voor negentien genotypen.

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Samenvatting

Toetsing van sla op resistentie tegen Myzus persicae

In augustus 1973 en mei 1974 zijn 645 PIVT-nummers uit de IVT-slagenenbank in de kas getoetst op resistentie tegen de bladluis *Myzus persicae*.

Voor het opsporen van resistentie is een methode ontwikkeld om in korte tijd grote aantallen genotypen te kunnen verwerken. Deze macrotoets berust op een uniforme verdeling van een bladluispopulatie, ontstaan na kunstmatig blootstellen aan bladluisaantasting, over de te toetsen planten en op een snelle en betrouwbare schatting van de aantallen luizen per plant ongeveer vier weken na het opbrengen van de luizen (Fig. 1).

Zowel in 1973 als in 1974 zijn significante verschillen in resistentie gevonden (Tabel 1). Het aantal bladluizen varieerde in deze jaren van 14 tot 74 resp. van 10 tot 83 per blad. De betrouwbaarheid van de macrotoets werd onderzocht door bij negentien genotypen met een verschillend resistentieniveau zowel tellingen als schattingen uit te voeren (Tabel 2, Fig. 3, 4 en 5).

Voor het uitvoeren van een genetische analyse en een praktisch gericht veredelingsprogramma is het noodzakelijk de resistentie van individuele planten kwantitatief te kunnen bepalen. Hiervoor is een microtoets ontwikkeld waarbij een aantal voor de populatiegroei bepalende factoren, zoals larvale gewichtstoename (biomassa) en larvenproduktie gedurende 4–7 dagen, als resistentiecriteria fungeren. De luizen worden bij deze microtoets in eenvoudige, goedkope en gemakkelijk hanteerbare klemkooitjes op het blad geplaatst (Fig. 2). Zowel tussen genotypen alsook tussen en binnen planten van eenzelfde genotype werden significante verschillen geconstateerd (Tabel 3, 4, 5, 6 en 7).

De macro- en microtoets zijn met elkaar vergeleken op negentien geselecteerde

genotypen met een verschillend resistentieniveau (Tabel 8). De resultaten van beide methoden komen goed overeen zoals blijkt uit de goede correlatie tussen biomassa na 7 dagen en het aantal bladluizen per blad ($r = 0{,}80$) en tussen de aantallen luizen en netto produktie over 4 dagen ($r = 0{,}83$) (Fig. 6 en 7). Ook de correlatie tussen biomassa en netto larvenproduktie was sterk ($r = 0{,}94$) (Fig. 8).

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