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RESISTANCE IN *CUCUMIS SATIVUS* L. TO *TETRANYCHUS URTICAE* KOCH. 2. DESIGNING A RELIABLE LABORATORY TEST FOR RESISTANCE BASED ON ASPECTS OF THE HOST-PARASITE RELATIONSHIP

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SUMMARY

The relationship between the twospotted spider mite and cucumber has been studied on plants and on leaf disks of a number of varieties with different levels of resistance. Existing laboratory tests are critically discussed and it appears that they are only reliable if many factors are taken into account. A new, more efficient laboratory test for resistance, measuring acceptance and reproduction is described.

INTRODUCTION

Reliable and efficient test methods are prerequisites to any resistance breeding program. Observations on successful field tests after natural attack or artificial inoculation might provide the most reliable data on differences in resistance. But field tests often fail because many factors influencing the development of a pest are uncontrollable in the field as has been demonstrated by VAN MARREWIJK & DE PONTI (1975).

If great differences occur between resistant and susceptible plants and the resistance is simply inherited, field tests may satisfy in spite of their shortcomings. But if the resistance is complicated in its appearance and inheritance, there is an urgent need for more sophisticated test methods. Such artificial tests, which are mostly executed under controlled environmental conditions in the glasshouse or laboratory, allow the detection of small differences in the level of resistance. This enables:

1. separating a complex resistance into its various components;
2. unravelling the inheritance of the resistance;
3. increasing the level of resistance by the accumulation of resistance genes.

Although artificial tests are often preferred, field tests should remain part of each research on resistance to confirm the results of artificial tests.

In developing artificial tests one should give permanent attention to a high correlation with the field situation (GUTHRIE, 1975; KOGAN, 1975; MOREAU & GAHUKAR, 1975; VAN MARREWIJK & DE PONTI, 1975). These tests must also be efficient so that many plants can be screened in a short period. If too laborious for routine breeding, artificial tests may still prove useful in the research phase of a breeding program by giving a better insight into the different aspects of the resistance.

A close study of the host-parasite relationship and the influence of the environment on it, preferably on susceptible and resistant varieties, should provide the basis of artificial tests. In this study many sources of variation affecting the relationship can be distinguished, such as:
1. the physiological condition of the plant;
2. the environment during the test;
3. the condition of the insect or mite used as inoculum;
4. differences in resistance.

In measuring differences in resistance the sources of variation mentioned under 1 to 3 can act as disturbing factors, the influence of which must be limited by standardization and good experimental design.

ARTIFICIAL TESTS FOR RESISTANCE TO TETRANYCHUS URTICAE KOCH

In the literature on resistance to the twospotted spider mite, *Tetranychus urticae* KOCH (reviewed by VAN DE VRIE et al., 1972 and DE PONTI, 1977), reference is frequently made to the use of artificial tests. There is, however, very little knowledge of the reliability of these tests and therefore it is astonishing to find that they are used on a rather large scale.

The aim of the present study is to reduce systematically possible shortcomings as far as testing for resistance in cucumber is concerned. Hereafter many aspects of a standard laboratory test, developed in our institute and to be outlined at the end of this paper, will be subjected to investigation.

Conditioning plant material and environment. Besides its level of resistance, the suitability of a plant as a host for insects and mites depends largely on its physiological condition, as affected by age, nutrition, turgor, treatments with agricultural chemicals and the environment. The environment also directly influences the development of insects and mites. The abundant literature on these subjects has been reviewed by VAN EMDEN (1966a, 1966b), SINGH (1970) and specifically for spider mites by VAN DE VRIE et al. (1972) and SUSKI & BADOWSKA (1975). In most studies on resistance to spider mites plant material and test environment are well described and often standardized.

The cucumber plants used in our experiments are grown in a glasshouse in accordance with common practice. Seeds are sown in trays containing sandy soil and after four days the plants are transplanted in pots filled with peat-soil of a very homogeneous and constant composition. About 12 days later the plants have one to two true leaves and are suitable for use in experiments. Neither pesticides nor fertilizers are used.

The experiments are carried out in rooms conditioned for temperature (26°C), relative air humidity (50–70%), daylength (14 hours) and light intensity (12000 mW/m^2). This environment is suitable for the growth of cucumber plants as well as for the development of twospotted spider mites. The pots are placed on benches, on a cotton blanket saturated with water to ensure an even water dose. In each experiment ten plants of a susceptible variety are included as standards.

Conditioning the inoculum. The twospotted spider mites used as inoculum in our experiments are from a population, which is representative for the Netherlands by repeated collecting on different crops throughout the country. This population is maintained in a glasshouse on susceptible cucumber plants, which are regularly replaced by young ones. The use of a definite laboratory strain, as practised by STONER & STRINGFELLOW (1967), CHAPLIN et al. (1968), GENTILE et al. (1969) and MACDONALD et al. (1972), has been rejected because it never contains the wide genetic variation of a natural population. Thus the risk of breeding varieties to only one particular strain is avoided. For similar reasons the mites are not reared in the laboratory, where the genetic composition and physiological quality of the population might change rather soon (GUTHRIE, 1975).

The initial reaction of an insect or mite to a new hostplant is often strongly influenced by the preceding host. This has been demonstrated for spider mites by BRAVENBOER (1959), SNETSINGER et al. (1966) and MATSUTANI (1968) and for insects by KENNEDY (1958), HOVANITZ (1969), YAMAMOTO et al. (1969) and HUBERT-DAHL (1975). Nevertheless, mites are mostly reared on beans no matter what crop one is studying. SCHUSTER et al. (1972), who studied resistance to the twospotted spider mite in cotton, alternate rearing on bean with rearing on cotton to avoid 'bean-specialization'. To be quite on the safe side we prefer to rear the mites continuously on cucumber, although there is no evidence for an influence of the preceding host on the sequence of a group of plants according to their level of resistance.

For most insects and mites reproduction is a function of age. To avoid irrelevant variation it is necessary to uniform the age of the mites to be used as inoculum. Different techniques were tried out.

1. Teleiochrysales are collected from heavily infected cucumber leaves. Although this technique is very accurate, it was rejected, because an unknown percentage of mites were – often invisibly – injured. Besides, the technique is too laborious.
2. Eight days before inoculation early in the morning an abundance of mites are shaken from heavily infected cucumber leaves on young non-infected plants. At the end of the day the mites are blown from the leaves with compressed air. The plants with eggs are placed in a controlled-climate room at 26°C and after 8 days the newly developed deutonymphs are used as inoculum. A disadvantage of this technique is, that individuals in quiescent stages are not removed by the air current. They will further develop and later contaminate the population of deutonymphs.
3. Another technique differs only in one detail from the preceding one. The mites are not removed from the leaves with compressed air, but killed with an acaricide without ovicidal action, like dichlorvos (DDVP). Thus the individuals in quiescent stages are also killed. It still has to be proved, however, that there is no latent influence of the acaricide through the eggs on the later stages.
4. The fourth technique is similar to the second except that it is carried out on large cucumber leaf disks (10 cm ⌀), which float on a 10 ppm solution of benzimidazol in water (DE PONTI & INGGAMER, 1976). A similar technique is used in RODRIGUEZ's laboratory (see DABROWSKI, 1972). But the question remains whether the deviating

Table 1. Acceptance and reproduction of deutonymphs reared according to three techniques. Figures followed by the same letter do not differ significantly from one another at the 5% level.

Rearing technique	Acceptance (%)	Reproduction (eggs/♀, 3 days)
on the plant, mites removed with air current	97ab	26.0a
on the plant, mites killed with DDVP	98a	26.2a
on leaf disks, mites removed with air current	91b	25.4a

physiological condition of leaf disks in comparison with intact plants influences the developing mites.

The quality of the deutonymphs reared according to the techniques mentioned under 2, 3 and 4 has been investigated. With deutonymphs of these 3 origins a standard acceptance and reproduction test, which will be described in detail at the end of this paper, has been carried out on 3 × 20 plants of a susceptible cucumber line.

The results, which are shown in Table 1, indicate that the quality of deutonymphs is almost identical. Only after rearing the mites on leaf disks is the degree of acceptance significantly lower. Mainly for the sake of efficiency the number two technique is preferred. The females thus reared are not fertilized, but according to BRAVENBOER (1959) this does not affect their reproduction capacity.

Investigations on measuring differences in reproduction. For measuring resistance, acceptance and reproduction must both be taken into account (DE PONTI, 1977). First the reproduction factor will be analyzed, while the acceptance factor will be dealt with later.

Until now resistance to the twospotted spider mite has been investigated almost exclusively by measuring reproduction of the mites or by damage ratings of the plants. In artificial tests the suitability of plants for the mites is mostly determined by measuring the reproduction during 24, 48 or 72 hours after inoculation. Sometimes all developmental stages of the progeny of the inoculum are watched, but mostly the observations are restricted to the eggs. In view of the earlier mentioned influence of the preceding host one might wonder how far the differences thus found are caused by the substrate tested.

To discover the most representative sample of the reproduction of this mite on cucumber we followed for one generation the entire reproduction of individual mites on eight varieties with a different level of resistance (as demonstrated by KOOISTRA, 1971). When the plants were in the third leaf stage one day-old female mite was placed in a 10 mm (⌀) leaf cage on the lower side of the first leaf (Fig. 1). After three days the number of eggs was counted and the mite and cage transferred to the next leaf. This was repeated every three days until natural death of the female. Thus the entire reproductive phase of the mites was subdivided into successive three-day periods. The brood was prevented from leaving the respective leaves by smearing the petioles with 'Tanglefooth' (Fig. 2). Seven days after the counting of the eggs the egg viability and larval mortality, further abbreviated to preadult mortality, were assessed.

The results are shown in Table 2. Analyses of variance for oviposition and net reproduction (= oviposition minus preadult mortality) have been executed followed

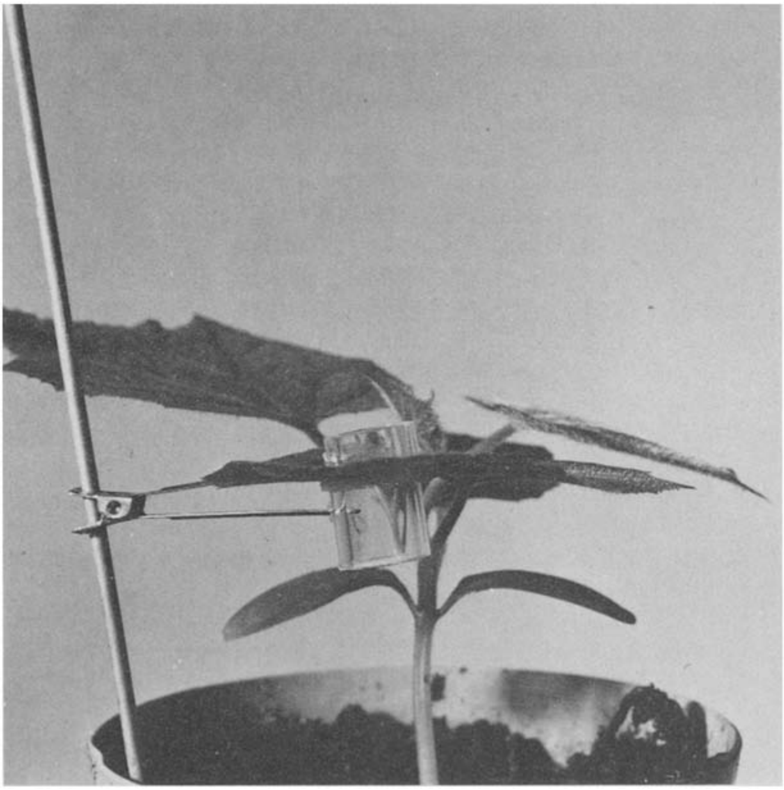

Fig. 1. Cucumber plant with leaf cage on the lower side of the first leaf.

by the multiple range test of KEULS (1952). Differences in preadult mortality, expressed as a percentage of oviposition, have been tested by means of binomial probability paper (FERGUSON, 1956). Only the first four three-day periods have been analyzed, since for practical reasons, later periods are less suitable citeria of resistance.

The results demonstrate that differences in host-suitability are expressed by differences in longevity, oviposition and preadult mortality, resulting in differences in net reproduction of the mite. The sex ratio also influences the rate of reproduction, but whether this ratio is affected by varietal differences has not yet been investigated. Although there are clear differences in longevity on the various varieties, this factor is less suitable as a criterion for resistance, because it requires daily observations for a long period of overlapping mite-generations.

The most complete information on varietal differences is provided by the total net reproduction. To what extent partial observations of oviposition or net reproduction are representative has been examined by comparing results of such observations with the total net reproduction. In most periods analyses of variance revealed significant differences, but the multiple range tests show that the degree to which the varieties can be distinguished from one another, concerning oviposition as well

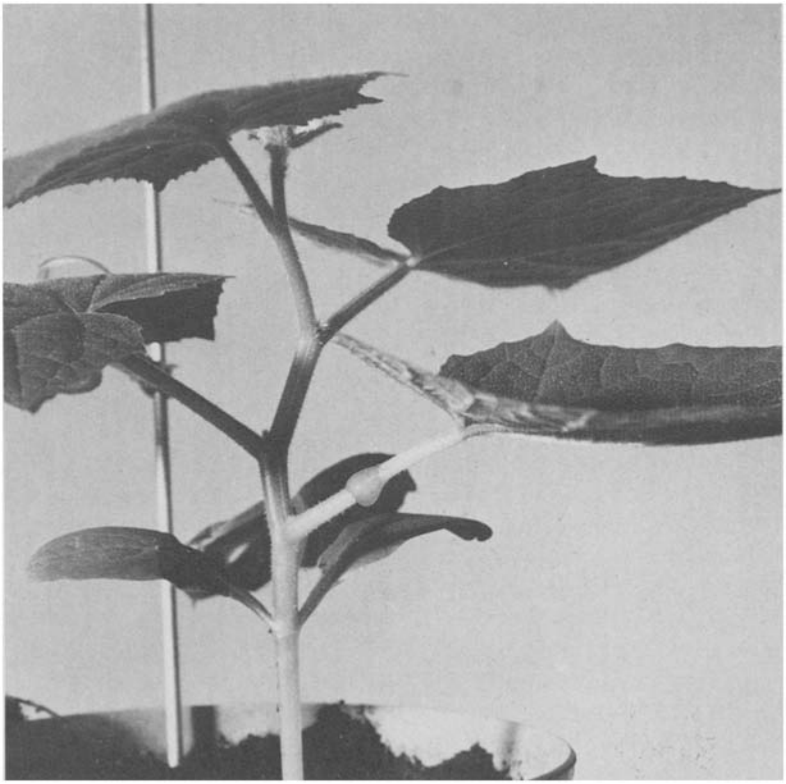

Fig. 2. Cucumber leaf of which the petiole is smeared with 'Tanglefooth'.

as net reproduction of the mites, differs markedly per period. The oviposition during the first few days – a widely used criterion of resistance – hardly gives any information on varietal differences (see periods 1 and 2). This is probably an aftereffect of the preceding host.

If for reasons of efficiency the observations have to be limited, the above analyses indicate that oviposition or net reproduction should be recorded in the third or fourth three-day period. Further information on this point can be obtained from the correlation coefficients between the total net reproduction and the net reproduction and oviposition over the successive periods (Table 3). These correlation coefficients have been calculated between plants, within each variety as well as within all varieties together, exluding the variety effect. Judging from these correlation coefficients the fourth period seems to be the better one.

There appears to be a very high correlation between the total net reproduction and the total oviposition. Although in an absolute sense the contribution of preadult mortality to the reproduction is quite large, as can be seen from Table 2, this high correlation suggests that the preadult mortality hardly affects the relative differences, the key factor for selecting resistant plants.

Although the discrimination between the varieties if based on oviposition is less

Table 2. Reproduction factors of females of *Tetranychus urticae* on cucumber varieties with different levels of resistance, recorded per period of three days. Figures followed by the same letter do not differ significantly from one another at the 5% level.

Variety	Number of plants	Oviposition/♀, 3 days, plant											Average longevity (days)
		Total	Period 1	2	3	4	5	6	7	8	9	10	
1. Granex	28	183a	33a	33a	29ab	26a	22	16	12	7	3	2	24
2. Sporu	17	163ab	31ab	31a	30a	25a	18	12	9	5	1	1	21
3. Varamin	16	157ab	30ab	29a	28ab	23ab	17	12	9	5	3	1	23
4. Német Kigyó	12	142bc	27ab	27a	26ab	24a	16	12	6	3	1	0	20
5. Taipei no 1	12	136bc	29ab	31a	24ab	19abc	16	11	4	1	1	0	20
6. Nobit	12	126bc	25b	32a	22b	16c	12	8	4	4	2	1	19
7. Hybrid L.G.P.	12	115c	30ab	30a	22b	16bc	9	6	1	1	0	0	17
8. Kecskeméti	17	115c	28ab	27a	27ab	19abc	8	4	2	0	0	0	16
		Preadult mortality/♀, 3 days, plant (%)											
1. Granex		3a	3a	3a	3a	3a							
2. Sporu		10b	6ab	12cd	10b	12b							
3. Varamin		10b	7ab	8bc	12b	13b							
4. Német Kigyó		12c	3a	7ab	14b	21c							
5. Taipei no 1		12bc	13cd	15d	12b	8ab							
6. Nobit		9b	9bc	8bc	10b	7ab							
7. Hybrid L.G.P.		25d	21e	26e	26c	29cd							
8. Kecskeméti		30d	17de	26e	39d	40d							
		Net reproduction/♀, 3 days, plant											
1. Granex		176a	32a	32a	28a	25a	22	15	11	7	3	1	
2. Sporu		145ab	29ab	27ab	27ab	22ab	17	11	8	4	0	0	
3. Varamin		142ab	28ab	27ab	25abc	20ab	16	11	8	4	3	0	
4. Német Kigyó		124b	26ab	25ab	22abcd	19ab	13	9	6	3	1	0	
5. Taipei no 1		119bc	25b	26ab	21bcd	17abc	14	10	4	1	1	0	
6. Nobit		116bc	23b	29a	20cd	15bc	11	7	4	4	2	1	
7. Hybrid L.G.P.		85cd	23b	22b	16d	11c	7	4	1	1	0	0	
8. Kecskeméti		79d	23b	20b	16d	11c	5	3	1	0	0	0	

than if based on net reproduction, it appears justified to restrict the observations to oviposition. In that case the fourth three-day period is probably the most effective for measuring differences.

In the above experiment, of each variety a number of plants were tested with one mite per plant. The varieties are genotypically rather homogeneous. The variation within the variety must therefore mainly be due to the variability of the inoculum in addition to random variation. Given this variability one should learn how many plants per variety or how many mites per plant must be used to be able to distinguish differences in resistance of a certain magnitude. This can be determined by means of the nomograph for the power of the F-test of FERGUSON (1962). Table 4 is an abstract of this nomograph. In the above experiment the coefficient of variation in oviposition during the first period was about 20%. The maximum difference between the varieties was 15%. This implies that at least 20 repetitions are necessary to distinguish

Table 3. Correlation coefficients between the total net reproduction and the Net Reproduction (NR) and Oviposition (O) of successive periods of 3 days and the total oviposition.

Variety	Correlationcoefficients (× 1000)								
	NR. 1	NR. 2	NR. 3	NR. 4	O.1	O.2	O.3	O.4	O. total
Granex	326	499**	697**	717**	339	526**	716**	704**	979**
Sporu	48	621**	618**	840**	−93	553*	518*	777**	973**
Varamin	577*	634**	426	853**	492	362	154	790**	937**
Német Kigyó	308	856**	756**	682*	361	891**	812**	835**	951**
Taipei no 1	467	720**	757**	901**	173	491	509	869**	928**
Nobit	−124	122	531	652**	−392	118	386	647*	986**
Hybrid L.G.P.	329	775**	664*	805**	−29	662*	645*	848**	794**
Kecskeméti	702**	680**	729**	855**	504*	578*	502*	738**	907**
All 126 plants together, variety effect excluded	306**	589**	633**	776**	201*	517**	538**	754**	949**

* $P \leq 0.05$; ** $P \leq 0.01$.

this difference with a probability of 50%. Although in the fourth period the coefficient of variation was larger (about 30%), the maximum difference is 40%, meaning that about eight repetitions are sufficient. This is another indication that testing should preferably take place in the fourth period in stead of in the first.

The importance of the preadult mortality is demonstrated by the fact that the differences between the varieties for net reproduction are much larger than for oviposition. One could also consider using the preadult mortality as an independent criterion for resistance. Whereas the oviposition in the first three-day period, as shown, hardly differs between varieties, the proportional preadult mortality (Table 2) of this brood is very divergent and the differences are in close agreement with the differences in total net reproduction. This confirms the experience of KENNEDY (1958), that the suitability of a hostplant should preferably be measured on the first progeny of an insect on its new host and especially on preadults, which are most sensitive to inadequacies. Although the preadult mortality over the first three days seems to be a reliable criterion for resistance, this has not been further examined, because its determination is too laborious in comparison with other criteria.

Table 4. Power of the F-test for two coefficients of variation (s/m) with several repetitions and at different probability levels (β) according to FERGUSON (1962) at a significancy level of 5%.

Number of repetitions	Distinguishable differences (%)					
	s/m = 20%			s/m = 30%		
	β = 0.5	0.7	0.9	β = 0.5	0.7	0.9
5	32	40	50	49	60	75
8	24	30	37	37	45	55
14	19	22	28	28	33	41
20	15	18	22	23	27	34

The above experiences on the importance of various reproduction factors as parameters for resistance only partly agree with those of DACOSTA (1971) and DACOSTA & JONES (1971). Concerning the preadult mortality the conclusions are similar, although we never found mortality figures up to 99%. This might be due to a difference in technique, because Dacosta transferred young larvae, which were hatched on a susceptible hostplant, to the testplant, whereas in our experiments larvae were born on the testplant. The latter technique seems better adapted to the natural situation, because larvae are not very mobile. The present study does not confirm Dacosta's statement, that differences in resistance are not expressed by differences in oviposition. A disagreement, which might be caused by the difference in varieties tested, but most probably by shorcomings of Dacosta's test, which have already been indicated.

The reliability of testing on leaf disks. In testing homogeneous varieties or breeding lines repetitions can be realized by using a number of plants. In a segregating population repeated observations on the reproduction of mites can only be made by using a number of leaf cages or leaf disks per plant. To economize labour the latter technique is to be preferred (DE PONTI & INGGAMER, 1976).

The behaviour and reproduction of insects and mites on excised plant parts, like detached leaves and leaf disks, may differ from the normal pattern on an intact plant (BECK, 1956; MÜLLER, 1958; LE BERRE, 1967; STORMS, 1969; MOREAU, 1971 and VAN EMDEN & BASHFORD, 1976). Nevertheless, leaf disks have been used on a large scale to determine differences in reproduction of the twospotted spider mite(RODRIGUEZ, 1953; DABROWSKI et al., 1971; MACDONALD et al., 1971; DABROWSKI. 1972; TULISALO, 1972; SOANS et al., 1973a, 1973b; SCHALK et al., 1975).

In our opinion the reliability of the leaf disk technique should be investigated thoroughly. For this purpose the reproduction of the twospotted spider mite on intact plants and on leaf disks of four cucumber varieties with a different level of resistance has been compared on 5 to 10 plants per variety. When the plants were in the second leaf stage one day-old female mite was placed in a leaf cage on the lower side of the first leaf and one on a leaf disk from the same leaf. The disks were put on filterpaper in a plastic tray with the lower side upwards according to the technique described by DE PONTI & INGGAMER (1976). After three days egges were counted, the 'plant-mite' and cage were moved to the second leaf and the 'disk-mite' to a disk from this leaf. This was repeated three times. After seven days the preadult mortality was observed.

Figure 3 shows the results of oviposition and preadult mortality, expressed as a percentage of oviposition. Oviposition and preadult mortality of mites on leaf disks do not differ significantly from those on intact plants provided they are not kept there for longer than three days. In case of a continued stay on leaf disks oviposition and preadult mortality become significantly lower. So testing on leaf disks appears to be justified only for short tests. This has been further examined in the following experiment.

On 15 plants of five varieties with a different level of resistance twenty day-old female mites were placed on the first leaf, of which the petiole was smeared with 'Tanglefooth'. After eight days 2 or 3 of these mites were placed individually in leaf cages on separate leaves and the same numbers of mites were placed separately on

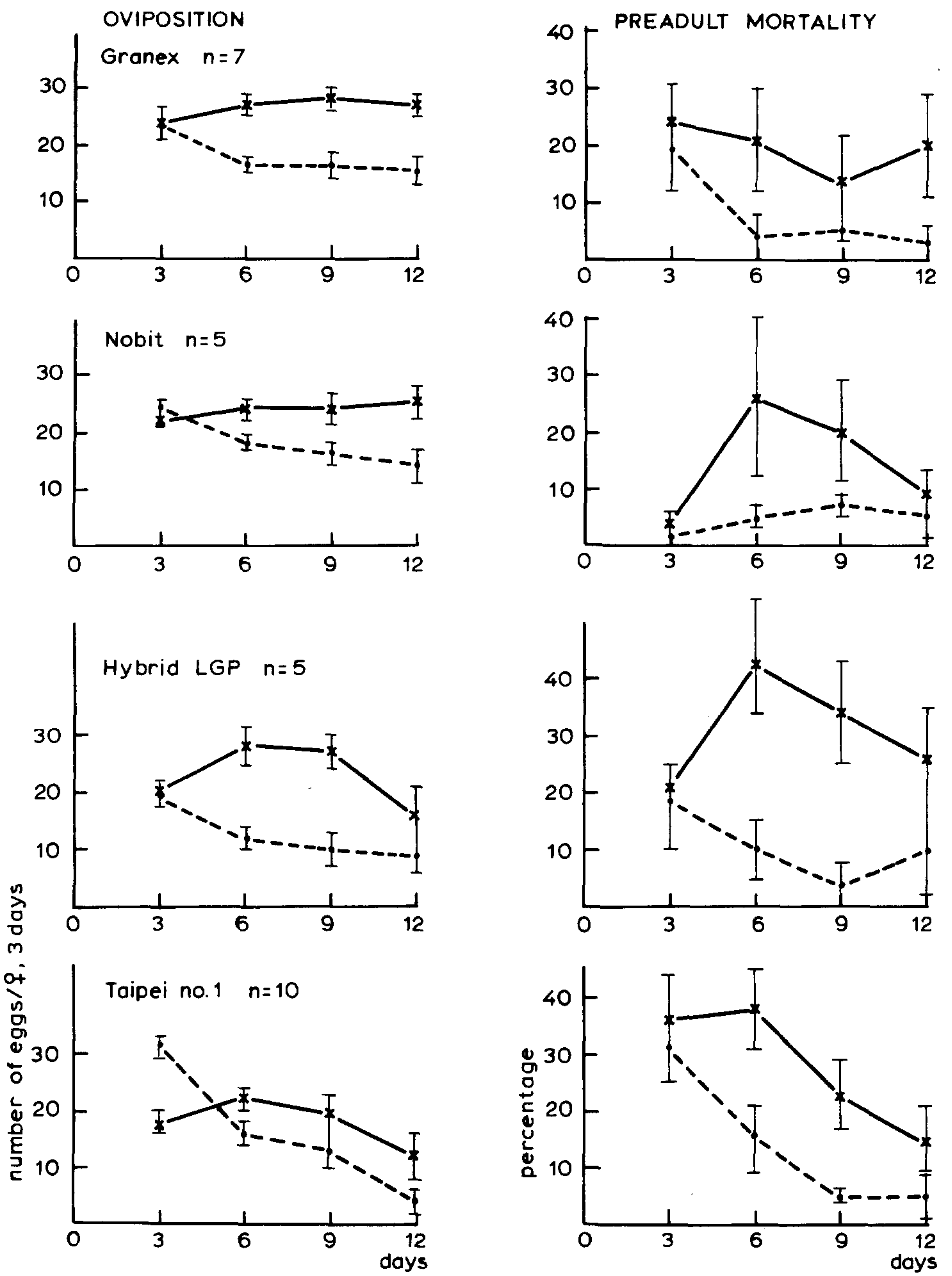

Fig. 3. Comparison of oviposition and preadult mortality per female per period of three days on intact plants (×) and on leaf disks (●). The vertical lines mark the standard deviation of the mean.

Table 5. Comparison of oviposition and net reproduction on plants and on leaf disks. Figures followed by the same letter do not distinguish significantly at the 5% level.

Variety	Oviposition		Net reproduction	
	plant	disk	plant	disk
Granex	27a	22a	25a	19a
Taipei no 1	20b	15b	16b	12b
Varamin	17bc	13bc	12bc	9bc
Hybrid L.G.P.	13c	9c	11c	7c
Kecskeméti	8d	11bc	6d	8bc
Mean	17.2	14.0	14.1	10.1

leaf disks from the same leaves. After three days oviposition and seven days later the preadult mortality were assessed.

The results, which are presented in Table 5 and Fig. 4, show a consistent change in the average level of oviposition and net reproduction. The purpose of a laboratory test can, however, only be to determine relative differences in resistance. Analyses of variance show that testing on leaf disks in this respect is about as informative as testing on intact plants in so far the comparison of varieties is concerned (Table 5). The same conclusion can be drawn from Figure 4, in which individual plants are

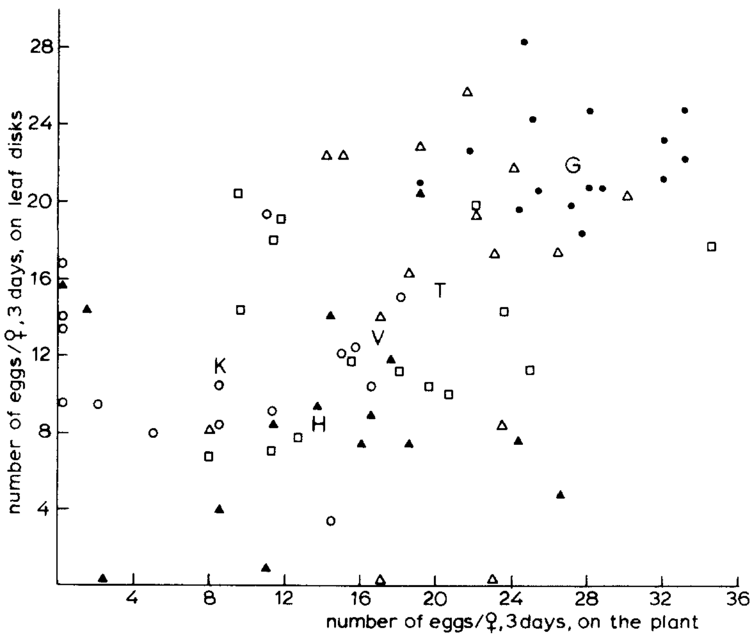

Fig. 4. Correlation between oviposition per female mite during three days on intact plants and on leaf disks from five varieties: ● 'Granex', △ 'Taipei no 1', □ 'Varamin', ○ 'Kecskeméti', ▲ 'Hybrid LGP'. The points represent averages per plant. The averages per variety are indicated by capital letters.

compared. The large variation between plants of one variety must mainly be ascribed to the variability of the inoculum, because the varieties are fairly homogeneous. The variation is extra large due to the fact that each plant was tested with rather few mites. In this connection it is worth noting that the variation on leaf disks is similar to that on intact plants.

It may be concluded from the above experiments that testing on leaf disks is justified provided that the tests are restricted to about three days.

DESIGN OF A LABORATORY TEST FOR RESISTANCE TO THE TWOSPOTTED SPIDER MITE IN CUCUMBER.

Based on the results of the above studies on various aspects of the relationship between cucumber and the twospotted spider mite, the following laboratory test for measuring differences in resistance was designed.

1. The plants are grown in a glasshouse according to common practice.
2. After two to three weeks, when the plants have a full-grown first leaf, they are moved to a controlled-climate room (26°C; 50–70% RAH; 14 hours light of 12000 mW/m^2).
3. The same day the petiole of the first leaf is smeared with 'Tanglefooth' and 20 female deutonymphs are placed on the leaf. Rearing the deutonymphs must be started eight days earlier.
4. After 10 days the remaining mites are counted (measure of acceptance).
5. Five disks are punched from the second leaf. On each disk one (nine day old) mite from the first leaf is placed.
6. Three days later the number of eggs is counted (measure of reproduction).

Although this test has been developed for cucumber, it might be adapted for any crop. Besides it might also be used in studies of the influence of non-genetical factors of a plant, like its nutrition, on the mites.

In contrast to glasshouse and field tests the above resistance test distinguishes two essentially different factors, viz. non-acceptance and reproduction, which together are a measure of resistance. This distinction does not mean that both factors are, of necessity, not interrelated. This question will be studied, before separate breeding for both factors is started. Measurement of the preadult mortality has been omitted from this test, because it does not essentially change the relative differences in resistance. With some extension, however, it can easily be assessed.

Testing plants for resistance to the twospotted spider mite by observing the oviposition of any mite on any leaf disk of any plant during some hours or days can hardly give useful information, as can be learned from the present study. This might partly explain, why after optimistic papers on differences in resistance based on such undefined tests, many projects have not been continued.

The research on which the above test is based indicates that also this test must be used with some reserve. The deviations from the natural situation and the large variation of the results may not be underestimated. As a consequence selection in a genetically heterogeneous population may not be too rigorous and the resistance of each selected plant must be confirmed by progeny testing before such a plant can be used for further breeding.

Before an ultimate decision about the reliability of this artificial test can be made, the correlation between this test and a field test has to be studied. This will form part of a following paper.

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