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GENETICS OF RESISTANCE TO ORGANOPHOSPHORUS COMPOUNDS AND ITS RELATION TO DIAPAUSE IN *TETRANYCHUS URTICAE* KOCH (ACARI)

Met een samenvatting: Erfelijkheid van resistentie tegen organische fosforverbindingen en haar verband met de diapauze bij Tetranychus urticae *Koch (Acari)*

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TABLE OF CONTENTS

1. INTRODUCTION

On many agricultural and horticultural crops spider mites of the genus *Tetranychus* will cause losses of great economic importance. The species of the *Tetranychus urticae* complex in particular are notorious pests, owing to their enormous speed of reproduction and their lack of particularity in the choice of the hostplant. These characteristics and the cosmopolitan distribution of the species have given rise to a most extensive survey.

The availability of modern synthetic insecticides shortly after the war added a new problem to the field of studies of the *Tetranychus urticae* complex: the development of resistance to these compounds.

Their great genetic variability enables the spider mites to give a ready answer to the acaricides, however varied the assortment. In many countries resistance developed to the extensive group of insecticides containing an organo-phosphorus compound as the active agent, as well as to more specifically working acaricides. In some areas resistance has even become a common appearance.

The resistance of insects of medical interest, such as *Anopheles*, *Aedes* and *Musca* has been intensively studied for many years (BROWN, 1960). Research into the resistance of agricultural insects has been done, however, on a far smaller scale. As the chemical control of spider mites threatens to develop into a neck-and-neck race between the chemical industry and the resistance-response of the mites, the desirability of an exhaustive investigation into biological backgrounds, the physiological and the genetical in particular, need to be stressed.

The present paper gives the results of a genetical approach of the resistance problem. To this end breeding and crossing experiments were performed with separate colonies of *T. urticae*, susceptible as well as resistant to organophosphorus compounds, in particular to parathion. In addition, it was attempted to throw some light on the genetics of resistance by means of experiments in which a combination was made between crossings of different strains and selections with insecticides.

It is known that in *T. urticae* the phenomenon of diapause occurs which is caused by photoperiod, in combination with temperature. The relation of the diapause phenomenon to the resistance is further pursued.

2. TAXONOMY, LIFE HISTORY AND BEHAVIOUR

2.1. TAXONOMY

The great variability in morphology has led to the description of numerous species and subsequently to a long list of synonyms of *Tetranychus telarius* (L.) (PRITCHARD & BAKER, 1955). Present interpretations, based on crossing experiments and morphological differences, led us to assume that within the *Tetranychus urticae* complex at least two species can be distinguished, viz.: *Tetranychus urticae* Koch and *Tetranychus cinnabarinus* Boisd. (BOUDREAUX, 1956; PARR & HUSSEY, 1960; VAN DE BUND & HELLE, 1960). In the field these two species can be distinguished by the colour of their female imagines: the active

summer form of *Tetranychus urticae* is yellowish green, whereas *Tetranychus cinnabarinus* is carmine coloured.

Since a practical solution of the problem of species within the *Tetranychus urticae* complex has not yet been found, the present article will adopt the designation *Tetranychus urticae* Koch, in accordance with a recommendation of the acarologists' symposium at Wageningen (1956). The names *T. telarius* (L.), *T. althaeae* (v. Hanst.) and *T. bimaculatus* (Harvey) can be considered as synonyms of *T. urticae*.

2.2. EGGS, ARRHENOTOKY

Data on the biology of *T. urticae* are numerous in literature. GASSER (1951) and LINKE (1953) give detailed descriptions; in the present paper essentials of the biology will only be dealt with, in case they are of importance to the research described.

SCHRADER (1923) distinguished two types of eggs in *T. urticae*, viz. with three or with six chromosomes, both types being viable. Also the larvae show the same difference in chromosomes; the nuclei of the spermatogonia contain three, those of the oögonia six chromosomes. SCHRADER concludes that the males are haploid and develop from unfertilized eggs, whereas females are diploid and proceed from fertilized eggs. This phenomenon, also found in other *Tetranychidae*, is called arrhenotoky.

The production of eggs is largely dependent on temperature, relative air humidity and food. BRAVENBOER (1959), amongst others, studied the relation between temperature and production of eggs, and found an optimum temperature of 30 °C. My experiments showed an average egg production during the life-time of a female of about 123 at 26 °C and a daily production of about 8 (VAN DE BUND & HELLE, 1960). BOUDREAUX (1958) stressed the important part played by the relative air humidity; he finds that the number of eggs produced in a dry atmosphere is twice the amount produced in humid surroundings. FRITSCHE (1959) made an extensive investigation into the influence of food on the production of eggs, and found that the sugar percentage of the leaves was of great importance.

2.3. METAMORPHOSIS

The development of the egg into adult is a typical epimorphosis (fig. 1), the various mobile stages (larva, protonymph and deutonymph) merging gradually and being interrupted only by resting stages (nymphochrysalis, deutochrysalis and teleiochrysalis resp.). The rate of development is determined by the same factors which govern the production of eggs, viz. temperature, air humidity and quality of food. At the optimum temperature of 30 °C the development of the eggs averages 3 days, whereas the post-embryonal development of the female takes 4 days.

Of importance is the phenomenon that there is a marked difference in speed of the post-embryonal development between males and females. If a mixture of haploid and diploid eggs of the same age is reared, adult males are present at a moment when the females are still in the teleiochrysalis stage. Often the males stand guard on a female chrysalis, in order to copulate as soon as she has cast

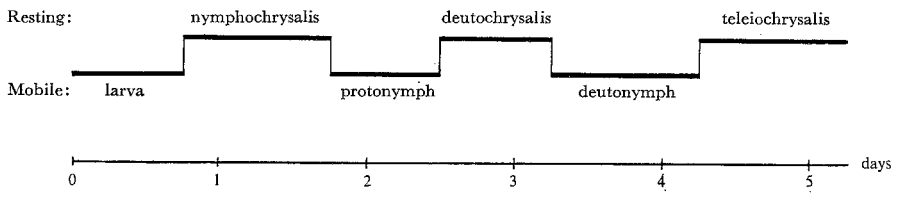

FIG. 1 Development of *T. urticae* at 25° C. The duration of each stage in days.

her skin. In a normal population, female adults are therefore always fertilized, in spite of a sex-index fluctuating between two and five. As a certain amount of eggs will remain unfertilized in a fertilized female, also males will develop in her progeny.

2.4. COPULATION

In some cases the males attempted to copulate with a female deutonymph or even with a teleiochrysalis. If a female after such a copulation is isolated, the offspring turns out to be male, consequently raised parthenogenetically. So these copulations had no results.

In advance to copulation the male crawls under the adult female, while the ventral side of the latter is resting on the male's dorsum. The male puts its first pair of legs behind the female's third pair and the second pair of legs clasps the female's tarsi. Then the male raises its opisthosoma and introduces the aedeagus into the genital opening, copulation taking 2 minutes on an average. Shortly afterwards the male starts looking for another teleiochrysalis. It also occurs frequently that a female, having only just cast her nymphal skin, copulates with various males. In general one single fertilization will suffice to provide a female with diploid eggs for the rest of her life, in combination with unfertilized haploid eggs.

2.5. SPREADING

Shortly after copulation the females will seek for fresh leaves. Thus females on bean plants will leave the lower leaves to migrate to upper leaves. This migrating tendency during the preoviposition period is of importance if for experimental purposes spider mites are cultivated on a single leaf, for in that case there is a great chance that young adult females will get lost when attempting to leave the leaf. It is therefore advisable to make provisions just before the last emergence to enable the young females to migrate to a fresh leaf. The pre-oviposition period is rather short at a temperature of 25–30 °C, usually less than 24 hours. After the pre-oviposition period, so after the production of the first eggs, the tendency to migrate decreases and will occur only under less favourable circumstances, such as shortage of food or overcrowding.

Owing to their light weight spider mites will readily spread; they are easily carried away on air currents. This mode of distribution will especially occur when the hostplants become overpopulated. Moreover, they frequently spin a thread, let themselves down with it and slight air currents can transport them

very easily. This renders a proper isolation of a spider mite population extremely difficult. Besides, because of their setae, they are easily transmitted by man.

2.6. HOSTPLANTS

The list of hostplants of the two-spotted spider mite is very long and no doubt this mite is the most polyphagous species of the family of *Tetranychidae* (ZACHER, 1921; LINKE, 1953). In addition to a practically unlimited number of wild plants, they are especially epidemic on culture plants.

2.7. HIBERNATION

In particular in the temperate zones hibernating possibilities of *T. urticae* are of great importance. As discovered by BONDARENKO (1950) adult females can enter into diapause. The diapausing females are to be distinguished from the yellowish green active summer form by a deep orange pigmentation. In the Netherlands the diapausing females appear in late summer and autumn.

At higher temperatures (20–30 °C) diapausing females are more or less active, but generally they do not take food, nor do they produce eggs. At low temperatures (below 15 °C) they often leave the hostplant to seek places to hibernate. Although the character of these places may differ widely, they always require protection from stagnant water. In a bean crop e.g., diapausing females are to be found in hundreds under the bark of the bean-sticks; on woody hostplants such as *Vitis*, *Buddleya*, *Sambucus* and *Rosa*, they crawl under the bark. On borderplants, such as *Verbascum*, they hibernate in hollow withered flower stems. In clay soil they crawl in corners and cracks of clods, where they often can be found in great numbers, enveloped by dense webs.

The cold hardiness of the diapause form during hibernation is a factor of considerable importance. Former experiments (VAN DE BUND & HELLE, 1960) gave an impression of the degree of cold hardiness. If diapause forms are protected from drying, they can be preserved at –2 °C for at least 8 months without any harmful effect. Besides the increased cold hardiness, the behaviour of the diapause form in winter must be considered: by crawling away they escape drowning, which is the fate of the active form dropping on the soil with the withered leaves.

2.8. DIAPAUSE

Diapause in *T. urticae* is induced by external factors. Photoperiod is of great importance, as well as temperature, and also food, though to a lesser degree. LEES' (1953b) fundamental study of diapause, mainly made of *Panonychus ulmi* (Koch), gives an insight into the relation of photoperiod and temperature, when inducing the diapause of mites. *T. urticae* is a typical long-day animal and hence a short-day period during development results in the diapause form. This effect, however, can be wholly or partially cancelled by high temperatures during the dark period. In fig. 2 we have shown a scheme indicating the critical area for a population subject to diapause.

As a rule the diapause can be terminated after a period of chilling. In this

TABLE 1. Termination of diapause in overwintering females of *Tetranychus urticae*, by exposure to low temperatures (after LEES, 1953a).

Temp. °C	Exposure for		
	50 days	75 days	100 days
1	3	85	97
10	1.5	44	87
19	0	0	–

way nature prevents diapause forms from becoming active prematurely on warm days in winter or early spring. LEES (1953a) gives the following data on the length of the period of low temperature (table 1).

However, from my own experiments it appeared, that even without a chilling period, part of the orange-coloured hibernating females show reversal to the active form. When bred from eggs at 18 °C and 12 h. photoperiod, 100 % of the obtained females showed the typical orange pigmentation. When these females were transferred to a place with a temperature of 25 °C, part of the winter form started sucking and at the same time the lateral side spots appeared, after which the orange pigmentation vanished gradually. These animals started also oviposition, but the speed of the reversal showed large individual differences: the pre-oviposition period varied from 2 days to 2 weeks. This partial reversibility from the winter form to the summer form has also been found in *Panonychus ulmi* (LEES, 1953a). The factors governing the reversal in *T. urticae*, are to be further investigated. All orange winter forms, however, even those coming to a rapid switch-over, were distinguishable from the active summer forms as regards cold-hardiness.

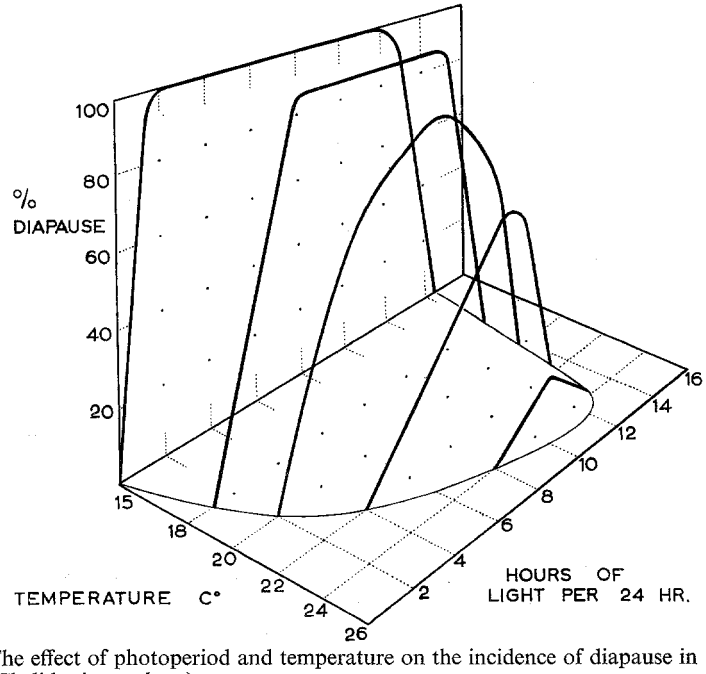

FIG. 2. The effect of photoperiod and temperature on the incidence of diapause in *T. urticae* (Chelidonium colony).

3. LITERATURE ABOUT RESISTANCE

3.1. DEFINITIONS

In applied entomology the term "resistance" is used in those cases, where an application of insecticides does not lead to the desired result.

The term 'resistance' therefore requires sharper limits. The W.H.O. Expert committee on Insecticides recommends the following definition (BROWN, 1958):

'Resistance to insecticides is the development of an ability in a strain of insects to tolerate doses of toxicants, which would prove lethal to the majority of individuals in a normal population of the same species.'

There is another 'resistance' which is often called 'natural resistance' and which is identical to *tolerance*. HOSKINS & GORDON (1956) define the term *tolerance* as follows:

'Tolerance provides a basic measure of ability to withstand a toxicant, with which changes in this ability can be compared. Tolerance may differ very greatly among various species, but as a rule does not vary much among representatives of a species living under natural conditions in different regions.'

In the following these definitions of resistance and tolerance will be used.

The tolerance of spider mites to an acaricide is influenced by a great number of factors and only few of them have been analysed. They may provide a simple explanation of an unfavourable result of an insecticide. FRITSCHE (1959) e.g. showed that the sugar contents of the leaf influences the tolerance to parathion which decreased if the mites took more saccharose. NEISWANDER, RODRIQUEZ & NEISWANDER (1950) demonstrated the part played by the hostplant. Temperature, air humidity, etc. are other factors influencing tolerance.

It is therefore advisable to use only quantitative laboratory methods to determine the degree of resistance of spider mites, and to test under conditioned circumstances. Field tests, with their many unverifiable factors, may give a misleading picture of resistance, especially as far as the level of resistance is concerned.

3.2. FIRST OCCURRENCE OF RESISTANCE

In the U.S.A., shortly after the first application of organo-phosphorus (OP) compounds in greenhouses, spider mites developed resistance. In 1947 and 1948 hexaethyl tetraphosphate (SMITH, FULTON, LUNG & BRIERLEY, 1947) and parathion[1]) (BLAUVELT & HOFFMANN, 1948) were used with great success for the control of spider mites. At various places in the Eastern United States resistant spider mite populations were found, mostly in rose greenhouses, as early as 1948 and 1949 (ENGLISH, 1950; GARMAN, 1950 and SMITH & FULTON, 1951). In 1950 a considerable number of rose greenhouses was found to harbour OP-resistant spider mites.

But also in other parts of the United States OP resistance impeded the fight against the spider mite. JEFFERSON & MACK (1953) reported resistance from California, GERHARDT & WENE (1959) recording a similar development in *Tetranychus cinnabarinus* Boisd. in Arizona.

[1]) 0,0-dimethyl (resp. diethyl) o-p-nitrophenyl thionophosphate.

The development of resistance in the United States did not confine itself to OP compounds. Compounds with a more specific acaricidal action such as aramite[1]), kelthane[2]), chlorobenzilate[3]) and tedion[4]) gave rise to resistance after repeated applications. The various publications of NAEGELE (1954, 1955 and 1956) and SMITH (1959 and 1960) show the complicated resistances of the spider mite.

In Europe papers on the occurrence of resistance in species of the *T. urticae* complex are particularly scarce, though in many countries OP-resistance has developed. It has been possible for me to obtain an impression of the distribution of OP-resistant *Tetranychus* species, by gathering written information from entomologists of various institues (table 2).

TABLE 2. Observations on resistance against OP-compounds in the two-spotted spider mite in Western Europe.

Locality	First observation	Source
France: Provence carnation and rose, inside and outside glasshouses	1951	L. DEPORTES Stat. Zool. Agricole, Antibes
Italy: Toscane carnation, in the field	1955/56	A. CAMPARINI Inst. Tecn. Agrar. Stat., Firenze
West Switzerland rose in glasshouse	1958	G. MATTHYS Stat. d'Essais Agricoles, Nyon
Belgium grape in glasshouse	1955	Stat. Agron. l'Etat, Gembloux
Southern Germany carnation in glasshouse	1952	DOSSE, (1952)
Netherlands: Aalsmeer rose in glasshouse carnation in glasshouse	 1950 1957	 v. MARLE (1951) HELLE (1959)
Denmark rose and cucumber in glasshouse	1951/52	A. KLOUGART Rolighedsvej 23 Copenhague
Norway rose in glasshouse	1951	FJELDDALEN & DAVIKNES (1952)

In most European countries OP-resistance is a problem, though mostly local. It is only of economic importance, when resistance is found to acaricides other than OP-compounds (HELLE, 1961), in rose, carnation and grape cultures in the Netherlands, France and Belgium.

From other parts of the world only sporadic information is available. MYBORGH at Stellenbosch (South Africa) reports a case of resistance in *Tetranychus cinnabarinus* Boisd. on apples (personal communication).

[1]) 2-(p-tert.-butylphenoxy)-1-methylethyl 2-chloroethyl sulphite.
[2]) 1,1-bis (p-chlorophenyl) 2,2,2-trichloroethanol.
[3]) ethyl 4,4-dichlorobenzilate.
[4]) 2,4,4', 5-tetrachlorodiphenyl sulphone.

3.3. DEGREE OF RESISTANCE

When the degree of resistance was determined, a high level OP-resistance has usually been found. WATSON & NAEGELE (1960) have found that their S 88 strain, selected with parathion, was 91 times as resistant as the control strain (as compared at the LD_{50}). Similarly the Cranbury strain of TAYLOR & SMITH (1956) is about a hundred times as resistant to malathion[1]) as a susceptible strain. BRAVENBOER (1959) finds for the resistant strain II the factor 20 and for strain III the factor 120. However, no absolute value should be placed upon the figures found for the resistance factor, as the methods of the various researchers are widely divergent. Moreover, according to VOSS (personal communication) the size of the resistance factor is to a large degree dependent on temperature.

SABA (1961) studied the development of TEPP[2]) resistance on five different hostplants. Selections for 12 generations gave several levels of resistance to TEPP, dependent on the hostplant. On *Humulus lupulus* the mites became highly resistant (LD_{50} increased to 220-fold), on *Phaseolus vulgaris* the LD_{50} increased only about 5-fold.

Exact data on cross-resistance (viz. resistance to another acaricide than the selecting one) are scanty, though from research on this subject (BRAVENBOER, 1959; GASSER, 1960 and SMITH, 1960) it could be concluded that a parathion resistance goes hand in hand with resistance to other OP-compounds as well.

In some cases the compound demeton[3]) forms an exception (BRAVENBOER, 1959). This may be explained by its systemic action, which may be rather complicated. When demeton is taken orally in *Megoura viciae* (Buckt.), the epithelial cells of the digestive tract will degenerate. When demeton is inhaled, these cells do not degenerate (VOSS & EHRHARDT, 1961).

The question may be asked, how the resistance level will react with relaxed selection pressure. This is of course only of scientific interest as far as laboratory colonies are concerned, in which any mixing with susceptible import-mites can be prevented. SMITH (1960) investigated this matter and described the resistant Cranbury-1 colony, which has been reared in the laboratory since 1949 and has maintained its resistance level for over 11 years (an estimated 500 generations) without any selection pressure. Also in our laboratory, the OP-resistant II colony has been reared since 1959 (about 100 generations) without any treatment with acaricides. In this case too there was no sign of any reversion of parathion resistance. The opposite was observed by DITTRICH (1961), who found that at a breeding temperature of 22 °C the resistance level dropped sharply within four months. At a temperature of 33 °C the mites were found to have practically lost their demeton resistance within three months (14 generations).

The change occurring in a mite population where a S- and a R-strain were placed on the same plants, was studied by LEHR & SMITH (1957). At the end of the experiment (91 days after mixing) the number of R and S mites was approximately the same.

3.4. CORRELATION OF RESISTANCE WITH OTHER CHARACTERS

Data exist on the correlation between resistance and other characteristics.

[1]) O,O-dimethyl S-(1,2-dicarboethoxyethyl) dithiophosphate.
[2]) Tetraethylpyrophosphate.
[3]) O,O-diethyl O-2-ethyl mercaptoethyl thionophosphate.

GASSER (1957) found, that at all temperatures a parathion resistant strain developed quicker at 90% R.H. than the normal strain. However, at a low R.H. (30–60%) and a temperature of 16–22 °C, the resistant strain developed slower than the normal strain. DITTRICH (1961) found numerous differences between a demeton resistant strain and a susceptible one. Decreased production of eggs, longer embryonal and post-embryonal developmental periods, less resistance to starvation, a decreased sucking activity and a shift of the sex index in favour of the male are listed by him as factors which may explain the reversion of the resistance.

Another aspect of a possible correlation may be found in the diapause pattern. Some OP-resistant spider mite populations were found to have an other critical area for diapause induction than susceptible populations (HELLE, 1961; SABA, 1961).

To what degree these facts are inherent to resistance, remains to be seen. Selections by treatments with toxicants may cause accidental characteristics, which owing to the isolation subsist in the population, but essentially may have no bearing on resistance.

3.5. GENETICS OF RESISTANCE

The genetic basis of resistance in the two spotted spider mite was first studied by TAYLOR & SMITH (1956). On the strength of reciprocal crossings between S & R spider mites (*Tetranychus bimaculatus* Harvey syn. *Tetranychus urticae* Koch) they concluded that the resistance to malathion is a dominant characteristic, and that resistance is transmitted by either sex, without maternal effects. In crossings between F_1 females of the R × S matings and haploid males from a resistant maternal parent, the backcross females were resistant. F_1 females of the S × R, crossed with haploid males from a non-resistant maternal parent produced backcross females which were resistant and non-resistant in a 1 to 1 ratio. Also males appeared to exist in this relation. This indicates that resistance to malathion probably is based on a single factor.

However, the argumentation of the monogenic character of the resistance shows a gap. TAYLOR & SMITH tried one single 'discriminating' dose to show the difference between S and R mites. Although resistance is a quantitative characteristic, it is desirable to use several doses for evidence of a Mendelian ratio. The possibility of an 'accidental hit' remains when working with a single dose, although the chance factor seems to be rather small, considering that the 1:1 ratio was found for males and females equally.

ANDRES & PROUT (1960) published a supposition of the same kind, with similar crossings between S and R strains of *Tetranychus pacificus* McG. Likewise they conclude from the 1:1 ratio in the backcross with S mites, that the resistance to parathion must be based on a single factor, though modifiers might enter into the picture.

DITTRICH (1961), on the strength of repeated backcrossings, attributes the resistance to polymery, as repeated crossings between R females and S males, using a weaker selection pressure, gave a decreasing resistance to demeton in later generations.

4. MATERIAL AND METHODS

4.1. REARING SPIDER MITE POPULATIONS

The various populations used for crosses were reared in special thermostat controlled cabinets. The temperature in each of these cabinets (size 150 × 50 × 50 cm) was kept at 25 °C, as a temperature of over 25 °C is injurious to the quality of the hostplant. The relative air humidity in each cabinet varied between 60 and 90%. The range of the chosen relative air humidity is a compromise: above 90% the egg production of spider mites shows a considerable decrease (BOUDREAUX, 1958) and at less than 50% the bean plants develop small, firm leaves, also leading to a smaller egg production. The level of the air humidity was maintained by an engine-driven air-transport system, driving the air through holes in the sides of the cabinets. These holes were covered with screening gauze of 50 μ mesh. Each cabinet was provided on the top and the rear side with double glass, so that long-day illumination by means of fluorescent lamps was possible. This prevented diapause of the spider mites. The amount of light in the middle of each cabinet was 2500 lux. No daylight was permitted to enter the system.

In each cabinet the spider mites were reared on young bean plants (*Phaseolus vulgaris* L.) grown in three zinc troughs (surface area each 80 × 40 cm). Crowding was prevented by replacing too heavily infested plants by fresh ones, which were infected by superposing a single infested leaf. In advance (in summer) these fresh bean plants were treated with methyl bromide in a fumigation room (40 ml methyl bromide per cu.m at 20 °C for 2½ hours).

For the transport of plants with spider mites for experiments, a hermetically closed transport container was used, exactly accomodating a zinc trough with bean plants. After opening the cabinets, the doors and the tunnel walls were scrubbed with a wet brush.

The spider mites for experiments with acaricides were reared from an 'egg-wave' in the cabinets, and kept separately from the culture proper by means of a water barrier. To this purpose young, later formed leaves were gathered from the culture. On these young leaves mainly adult females, migrated from the lower leaves, were present. Under a binocular stereomicroscope these females were removed, and only eggs remained on the leaves. The ages of these eggs may differ 4 days at the most, as the length of the egg stage is 4 days at 25 °C. These leaves with eggs were pinned on fresh plants, placed in a zinc trough in the cabinet. A water barrier prevented the intrusion to the rest of the same colony. On an average of 12 days after the start of the egg-wave, the mites were used. The limits of the ages of the adults thus obtained are to be calculated from the duration of the development of the eggs (4 days) and the post-embryonal development (6 days). The age of the adults will vary from 2 to 6 days.

The colonies reared in the cabinets for crossing experiments were:

CHELIDONIUM COLONY(S), from a spider mite colony on *Chelidonium majus* L., found near Lisse (Netherlands dune border district), in laboratory culture since May 1959. This colony was susceptible to parathion.

COLONY II (R) was gathered in a rose greenhouse at Aalsmeer (Netherlands). Here greenhouse roses have been grown and regularly treated with various OP-compounds since 1947. Since 1958 this population has not been treated

with toxicants in laboratory culture. It is highly resistant to parathion.

ANFAELLIG COLONY (S), found on *Phaseolus vulgaris* L. in a greenhouse at Hohenheim (Germany), received from Professor Unterstenhöfer[1]) in October 1960, was susceptible to parathion.

STRAIN SYSTOX (R) was selected for resistance with demeton out of strain Anfällig. These selections were effected in the biological laboratory of the 'A.G. Bayer' at Leverkusen (Germany). The initial selection pressure of demeton was such, that mortality varied between 50 and 70%. When a resistance level was reached after six generations, the strain was only occasionally treated with demeton. Shortly after the receipt of this strain in our laboratory in October 1960, it was sprayed with 500 ppm parathion. The strain has been reared since in our laboratory without further treatments with toxicants. Strain Systox was highly resistant to parathion.

4.2. REARING SPIDER MITES IN PETRI DISHES

For crossing purposes and also for diapause experiments, we made use of detached leaf cultures in a Petri dish. This Petri dish (diam. 8 cm) was filled with cotton-wool saturated with a nutrient solution, containing 213 mg KNO_3, 127 mg $MgSO_4.7$ aq., 141 mg KH_2PO_4, 5 mg $(NH_4)_2SO_4$, and 186 mg NH_4NO_3 per l. of tap water. A young bean leaf with an average surface of 25 sq.cm was pressed on the cotton-wool, the petiole in the cotton-wool and the tip of the leaf upwards. After some days the leaf will root in the cotton-wool. The leaf was pressed on the cotton-wool in such a way, that the surface of the leaf formed some niches. Mites placed on the leaves will find shelter places and thus will not spread into the cotton-wool.

The Petri dishes were placed in a glass box at 25 °C and received a long day illumination (2200 lux). A water barrier formed an extra isolation to prevent migration to other dishes.

4.3. EXPERIMENTAL PROCEDURE ON SPIDER MITES WITH ACARICIDES

Young bean plants, 7–8 days old, their first leaves forming an angle of 90° with the stalk, and in advance selected for strict uniformity (length of the plants 12 cm, leaf surface 25 sq.cm), were sprayed with the acaricide on a rotating disk. The spray nozzle was placed at a distance of 100 cm from the leaves and the spraying pressure was kept constant. Spraying was stopped when a drop formed on the tips of the leaves, the spraying time averaging 9 seconds.

After spraying the plants were allowed to dry at a temperature of 25 °C and then a small cage of plexiglass, 18 mm diam., was placed on each leaf (MELTZER, 1955). With the aid of a marten-hair brush ten adult females were placed in each cage. For each concentration of a series 6 × 10 mites were used. Mortality was checked after four days.

In most experiments adult mites of 2–8 days old were used.

For crossing experiments this method has the advantage, that the difference between individual susceptible and resistant mites is very large.

In most cases several experiments were made to determine the susceptibility

[1]) The author wishes to thank Prof. G. UNTERSTENHÖFER for sending live cultures of mites.

of a colony. The results of the various tests were drawn on log-probit paper and the mortality percentages were corrected with the mortality of the check according to Abbott's formula. [1])

4.4. THE CULTURE OF TEST PLANTS

As test-plant the variety 'Dubbele witte zonder draad' of *Phaseolus vulgaris* L. was used. This variety is characterized by having only a few, very soft hairs on the leaves and by a quick and regular growth. The beans were sown on sharp sand and cultivated at a constant soil temperature of 26 °C with an extra illumination of fluorescent lamps, giving a minimum illumination threshold of 2400 lux.

For the experiments exclusively plants of 7–8 days old were used, in which stage the second pair of leaves is still very small.

During the whole culture period the plants in the greenhouse were shaded against direct sunlight.

5. GENETIC ANALYSIS OF RESISTANCE TO ORGANO-PHOSPHORUS COMPOUNDS

5.1. INTRODUCTION

The most direct way of gaining insight into the genetic basis of OP-resistance is the crossing of resistant (R) with susceptible (S) mites.

The hybrids of *Tetranychus*-strains, however, present special difficulties. The main problem is to find strains, the hybrids of which will readily produce fertile eggs.

As described by various authors, crossings between species within the *Tetranychus urticae* complex as a rule produce sterile bastards. For instance crossings between *Tetranychus urticae* Koch and *Tetranychus cinnabarinus* Boisd. result in F_1-females producing only few eggs, if any. Moreover, most of these eggs will not be viable (BOUDREAUX, 1955; HUSSEY & PARR, 1958 and VAN DE BUND & HELLE, 1960). As a matter of fact this very crossing sterility supports the conception that these groups are separate species. Crossings within the species as a rule produce fertile offspring.

Likewise, it was found that the bastards of mutual crossings of certain *Tetranychus urticae* populations, though not sterile, were incompatible to a high degree, as the following example will show. In 1960 the colony 'Baardse' originating from an Aalsmeer (Netherlands) rose greenhouse was found to be OP and kelthane resistant (HELLE, 1961). Crossings between this colony and the S colony Chelidonium produced females in the F_1, characterized by a distinct red colour, whereas the parents were normally yellowish green. Moreover, these females lacked the dorsolateral spots. When isolated, these red females appeared hardly to suck and to produce only a few eggs. In the F_1 some normally coloured females with a higher viability were also found.

[1]) $\frac{a-b}{b} \times 100\%$, whereby a represents the number of survivors of the control and b the survivors of the object concerned.

Table 3 shows a record of these crossings. While the average daily egg production of the Baardse and Chelidonium colonies amounted to 3.0 and 5.0 respectively, the egg production of the F_1 of the reciprocal crossings was found to be 1.6 and 1.4. In addition the greater part of the eggs produced by the F_1 females appeared to be non-viable (viability 22 and 35% respectively). Ultimately the further offspring of these crossings largely collapsed. It is obvious that such incompatibility factors may seriously influence the results of genetic resistance tests, all the more so if quantitative data of the resistance are to be considered.

Partial incompatibility of the hybrid, though less pronounced, was also observed in two other colonies.

TABLE 3. Egg production and viability of crossings between Chelidonium and Baardse.

Cross	Generation	Pairs	Egg production					Sex Ratio ♀:♂
			Total 7 days	Mean per pair	Range	♀/day	%Mean viability	
Chel × Chel	P_1	10	353	35	15–54	5.0	93	4.1:1
Ba × Ba	P_1	10	211	21	11–33	3.0	88	4.0:1
Chel × Ba	P_1	10	411	41	16–62	5.9	79	3.1:1
	F_1	63	723	11	1–31	1.6	22	4.7:1
Ba × Chel	P_1	9	312	35	15–49	4.9	73	2.3:1
	F_1	56	552	8	0–43	1.4	35	2.7:1

In my experiments the hybrids of Chelidonium × II, as well as those of Anfällig × Systox appeared to be completely fertile. The percentage of non-viable eggs of these two crossings amounted to 10% in a single case, but was 5% on an average, which may be considered to be normal. The genetic work was therefore effected with these four strains.

Another difficulty of a more technical character is the question of feeding and isolation of the crossing progeny. In this respect *Tetranychus* is far more difficult to test than e.g. *Musca*. If the resistance of the offspring is to be tested, it should be reared under the same food conditions as in the normal culture, viz. on whole plants and not on leaves in Petri dishes. However, on whole plants it is difficult to rear age-waves, a requirement for the parathion resistance test of

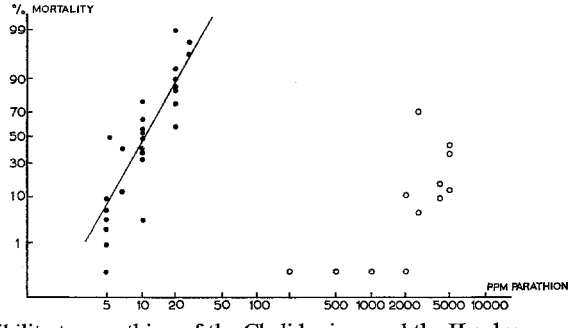

FIG. 3. Susceptibility to parathion of the Chelidonium and the II colony.
... = Chelidonium (9 experiments)
ooo = II (4 experiments)

the offspring. The isolation of mites on whole plants entails great difficulties and requires excessive space.

The solution of these two problems had to be a compromise. The crossings were effected on leaves in Petri dishes, producing egg-waves of known ages. After the larval development these leaves were transferred to whole plants, permitting thecomplete development of the mites in circumstances optimum to feeding. This mode of rearing the progeny of crossings appeared to be workable and capable of analysis. Fig. 3 and 4 show the susceptibility to parathion of the spider mite colonies involved in the crossings. The black spots indicate the percentages of mortality, corrected with the check mortality, and are the results of tests made on various dates. From these figures it appears that a single experiment can give information on the parathion tolerance of a colony, but that the spread of the individual dosis-mortality values, especially in the R colony, does not permit a reliable conclusion as to the LD_{50}.

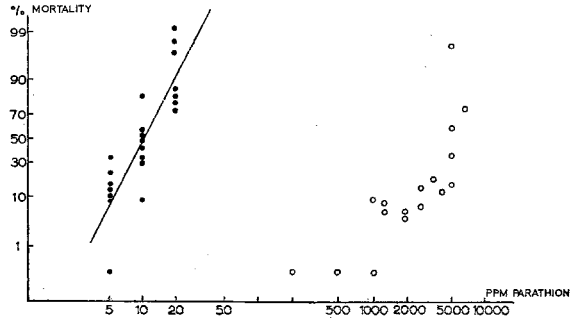

FIG. 4. Susceptibility to parathion of the Anfällig colony and the Systox strain.
... = Anfällig (7 experiments)
ooo = Systox (5 experiments)

In order to obtain an impression of the reliability, the results of a series of experiments with the Chelidonium colony were fed to a computer, and the LD_{50} were determined as well as the slope value of the ld-p line for each experiment (FINNEY, 1952). The results of the calculations are shown in table 4. In this table the LD_{50} is the median lethal dose in ppm causing an average mortality of 50% of the mites, and M is log LD_{50}, b is the slope value, viz. the angle of the ld-p line. The variations per test of M and b are indicated by sigma M and sigma b.

TABLE 4. Slope-value (b) and LD_{50} of the Chelidonium colony in a series of 8 experiments. M = log LD_{50}.

Experiment	b	σb	LD_{50}	M	σM
1	5.41	1.149	11.82	1.073	0.041
2	3.57	0.596	5.53	0.793	0.043
3	6.36	0.897	8.76	0.942	0.023
4	4.01	0.500	10.70	1.029	0.027
5	4.58	0.853	13.87	1.142	0.031
6	5.46	0.702	10.07	1.003	0.025
7	5.04	0.706	11.82	1.073	0.026
8	5.00	0.798	12.18	1.086	0.028

There are considerable variations in the slope value b and the LD_{50} in the various experiments. The slope value varies from 3.57 to 6.36 and the LD_{50} (average 10.59) in experiment 2 shows the exceptional value of 5.53 ppm.

This variation is not at all surprising, as experiments on living plants are seldom homogeneous. There will be many disturbing factors, in spite of a meticulous selection for uniformity of the plants, animals and conditioned circumstances.

When verifying the mortality, other factors appeared to have influenced the results of the experiments with the R colony. At concentrations of 4000 ppm parathion and more a repellent effect of parathion appeared. Many mites enveloped themselves in webs at the top of the cages and were thus isolated from contact with the parathion residue on the leaf. Another problem was, that parathion became phytotoxic at concentrations of 6000 ppm and over, making experiments with higher concentrations impossible. These circumstances caused a rather great fiducial interval of eventual ld-p lines of the R colonies.

However, the difference between S and R mites, at least when using the described method, is very large. The S mites were completely killed with 40 ppm parathion, whereas the R mites only showed measurable mortality at 1000 ppm. This easy discrimination between S and R mites is of particular importance in genetic experiments.

5.2. FIRST CROSS OF RESISTANT × SUSCEPTIBLE; DOMINANCE OF RESISTANCE

The first object was to investigate, whether also at our circumstances parathion resistance was inherited as a dominant factor and whether both sexes could transfer resistance to their offspring.

By means of a marten-hair brush a number of teleiochrysali of the S strain were placed on a bean leaf in a Petri dish. The dish was kept in a glass box for 24 hours at a temperature of 25 °C. During this period the chrysali completed their development into adult females; thus the sex of the teleiochrysali could be determined with certainty. Afterwards an excess of males of the R strain was added. In most cases the males started copulating within a few minutes after the transfer.

Two days later the males were killed and the females were transferred to a fresh leaf, again in a Petri dish. They remained on this leaf for three days, during which period many eggs were laid. Then the females were transferred to another fresh leaf in a Petri dish to produce a second batch of eggs.

In this manner egg waves were made in series with a maximum difference in age of three days. These eggs remained in Petri dishes until the first deutonymphs developed. Next the bean leaf was removed from the cotton-wool in the Petri dish and transferred to a young bean plant. The bean plant was placed in a glass case with a temperature of 25 °C and isolated by means of a water barrier.

This method of rearing provided in various stages an F_1 consisting of adult females of the same age-group. These females were used for the parathion susceptibility tests.

Results. The crosses between Chelidonium (S) and II (R) were effected by means of 23 Chelidonium females, whose offspring supplied the material for the parathion test. The mortality figures corrected with Abbott's formula are shown in table 5.

TABLE 5. Mortality percentages of the offspring of Chelidonium × II by exposure to several concentrations of parathion.

Concentration parathion	Number of females tested	Mortality after four days
6000 ppm	50	94%
5000 ppm	50	96%
4000 ppm	50	62%
2000 ppm	50	21%
1000 ppm	50	0%

Crosses between Anfällig and Systox provided a more convincing picture of the inheritance of the resistance. These crosses were made reciprocally, for which 58 Anfällig females and 65 Systox females were used. The results of the F_1 are shown in figures 5 and 6. In total 850 F_1 females of the cross Anfällig × Systox were used for a series of six experiments, and 960 F_1 females of the reciprocal cross were available for four experiments.

The LD_{50} is calculated at 2651 ppm and 2199 ppm for Anfällig × Systox and Systox × Anfällig respectively.

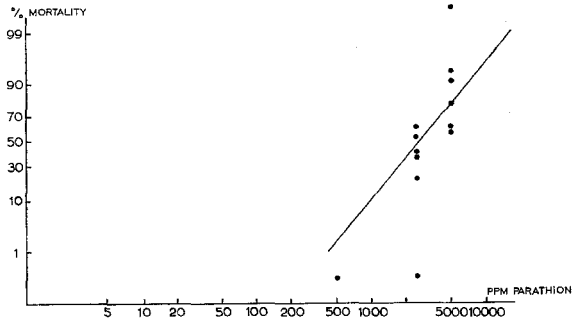

FIG. 5. Susceptibility of Anfällig × Systox (F_1) to parathion (6 experiments).

These experiments show clearly the dominant character of parathion resistance.

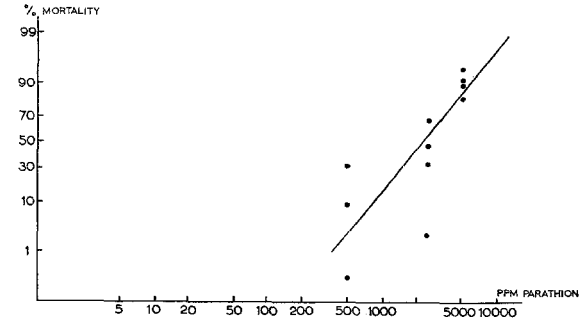

FIG. 6. Susceptibility of Systox × Anfällig (F_1) to parathion (4 experiments).

From the reciprocal cross between Anfällig and Systox the conclusion may be drawn, that females as well as males may transfer resistance. The difference in LD_{50} between Anfällig × Systox and Systox × Anfällig is not significant.

5.3. BACKCROSS (S × R) × S, AND THE EVIDENCE OF ONE MAJOR FACTOR

In theory there is a simple method of determining whether resistance to parathion in spider mites is due to one or more genetic factors. As the males are found parthenogenetical and haploid, the male offspring of a hybrid of R × S would be eminently suitable for an analysis of gametes. In practice, however, this is very difficult, because no suitable methods exist of experimentation with male spider mites. Owing to their small size they escape from their cages; if they are not confined, they will leave the plants.

Yet the haploidy of the males can be utilized in the analysis of gametes. The previously described experiments have shown that the resistance characteristics are dominantly inherited, and a backcross of the hybrid with an S mite is, therefore, the proper method for an analysis of the gametes. Starting from S females and R males in the P_1, only S males will be produced in the F_1. By removing the R males after copulation in the P_1, the backcross will be effected automatically with S males formed by parthenogenesis. Therefore no isolation of the F_1 teleiochrysali will be necessary. Starting from R females and S males in the P_1 complicates the matter owing to the appearance of undesired R males in the F_1.

Female teleiochrysali of the S strain were randomly selected for the two following experiments.

The males added after the complete development of the teleiochrysali were the offspring of a single unfertilized female of the R strain. They were permitted to remain with the S-females for two days, after which period they were destroyed.

From the eggs produced by the females the hybrids were reared. From these hybrids adult females were collected and placed on a bean leaf in a Petri dish.

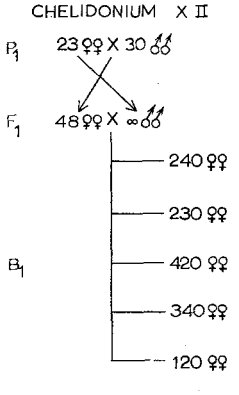

FIG. 7. Crossing scheme of Chelidonium × II. The numbers of females in the B_1 (first backcross) were used for susceptibility experiments.

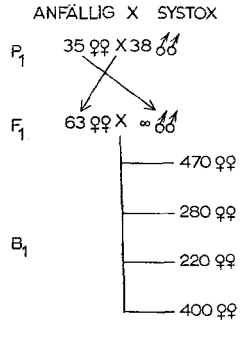

FIG. 8. Crossing scheme of Anfällig × Systox.

As described on page 165 egg waves were obtained with a maximum difference of age of four days. The mites (F_2) hatched from these eggs were again reared on whole plants in glass boxes at 25 °C.

A total of 48 F_1 females tested in the cross between Chelidonium and II produced an offspring of 1350 females, which were reared and used for experiments with various parathion concentrations. An F_1 of 63 females was the basis of the cross between Anfällig and Systox. The schemes are shown in figures 7 and 8.

Results. The mortality was corrected with Abbott's formula and traced on log probit paper. (fig. 9 and 10).

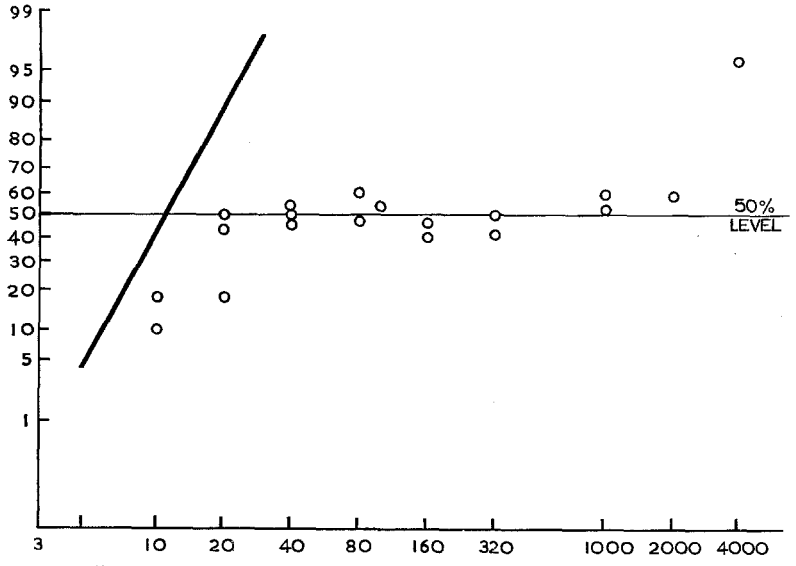

FIG. 9. Mortality percentages of the back-cross (Chel. × II) × Chel. The left ld-p line is of the Chelidonium colony.

The dots represent the mortality percentages of the B_1[1]) at various parathion concentrations. For purpose of comparison the ld-p line of Chelidonium is drawn in fig. 9. In fig. 10 the left ld-p line represents the susceptibility of the Anfällig-colony, the right line the susceptibility of the hybrid Anfällig × Systox.

It is most remarkable that the various parathion concentrations within the range of 50–1000 ppm always produce a mortality of about 50%, both for the B_1 of Chelidonium × II, and for Anfällig × Systox. Within this range an increased dosage does not produce higher mortality. This clearly indicates the presence of two categories, viz. susceptible and parathion resistant mites in a ratio of 1 : 1. In other words, the B_1 is split up into two genotypes. This can only be explained by attributing the parathion resistance to one major factor. If this factor is represented by A, in the B_1 the female genotypes aa (S) and Aa (R) will be present in equal numbers, which provides a natural explanation of the mortal-

[1]) B_1 = First backcross generation.

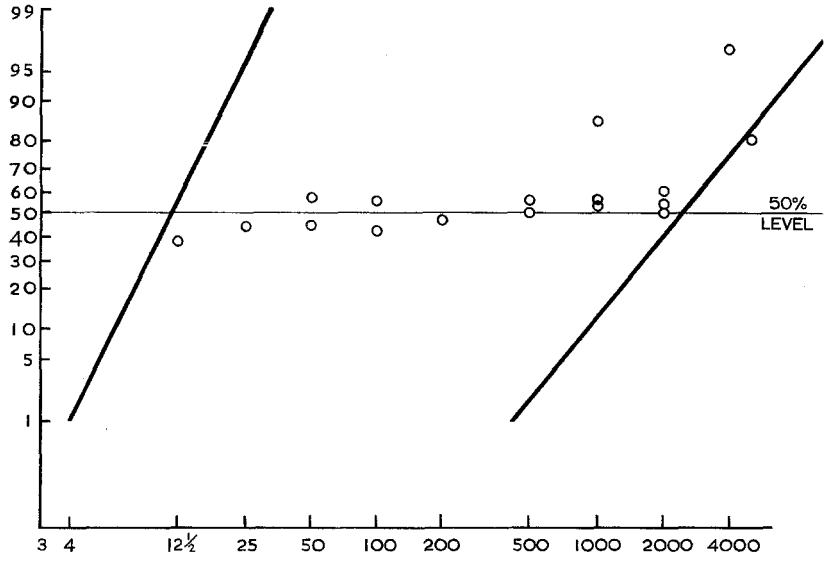

FIG. 10. Parathion susceptibility of the F_2 of Anfällig × Systox. The left and right ld-p lines are of Anfällig and the hybrid Anf. × Systox respectively.

ity data in figures 9 and 10. The results give no indications that other factors could have modified the effects of the parathion resistance up to a level of 1000 ppm.

These results are in accordance with those obtained by ANDRES & PROUT (1960) with *Tetranychus pacificus*. However, according to these authors, there is still room for the assumption that other genes play a part.

5.4. CROSS BETWEEN II AND SYSTOX

Crossing the hybrids of Systox × II with S mites, could provide an answer to the question, whether the resistance factor of II and Systox are identical. If the genes are in the same locus, the offspring will consist of resistant mites only. However, the experiment was not workable, as both the hybrids of Systox × II and those of the reciprocal cross were partly incompatible. No more than up to 20% females were found, characterized by a reddish tinge, producing a few eggs or none. Therefore the quantitative reliability of this alleles test did not meet the required standard.

5.5. REPEATED BACKCROSSINGS UNDER SELECTION PRESSURE; BREEDING OF STRAIN SP

CROW (1957) indicates the system of repeated backcrossings under selection pressure as a method of genetic approximation of resistance. This method of crossing enables the isolation of a major factor. Repeated backcrossings of the F_1 of R × S with S animals will substitute alleles from the S strain for minor, eventually vigour factors of the resistance complex. Due to the selection pressure, the more important R factors will maintain their position. If the backcross is

continued for several generations, the R factor will be assimilated to the S genome, and be incorporated in it. It will then be divested from possible modifying secondary factors, accidentally produced in the R strain by the method of selecting. Thus various resistances may be separated as has been demonstrated by BUSVINE (1953).

One of the objects of backcrossing was to investigate the level of resistance for which the factor A is responsible, because the first backcross (= gametes test) was not conclusive at that point. It was important to choose the selection pressure not too high since otherwise the possibility exists that other factors will be selected besides the factor A. If the selection pressure is taken too low, however, vigour factors may be introduced with the S genome. Most favourable is a concentration capable of killing all S mites. The ld-p line of Anfällig indicates that a concentration of 100 ppm parathion meets this requirement.

Experimental. Figure 11 shows the scheme of the crossings. A mass cross (10 pairs) between female Anfällig teleiochrysali and male Systox adults was effected on a bean leaf in a Petri dish. When the eggs were laid, the parents were removed. From these eggs the G_1[1]) was reared on leaves in a Petri dish. This generation consequently consisted of resistant females of the type Aa and a-males only. An egg-wave of this generation was produced on fresh leaves in a

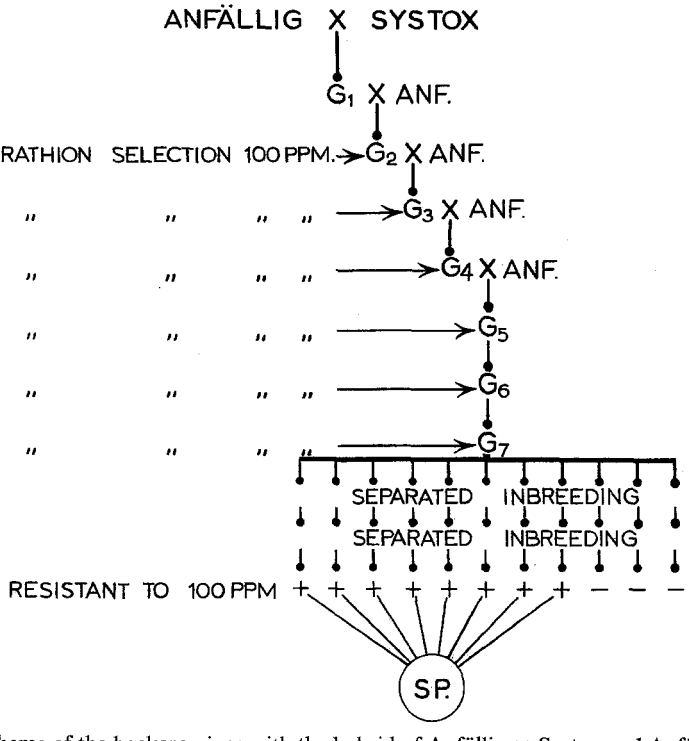

FIG. 11. Scheme of the backcrossings with the hybrid of Anfällig × Systox and Anfällig.

[1]) G_1 = first generation.

Petri dish which was reared to the teleiochrysalis stage of the females. Fifty of this female teleiochrysali were transferred to a fresh leaf, after which males of the Anfällig colony were added.

After a few days all adult fertilized females were placed in cages on bean plants, treated previously with parathion (100 ppm). The surviving mites were transferred to a bean leaf in a Petri dish to produce a new egg-wave. These eggs were once more reared to the teleiochrysalis stage, to be crossed again with Anfällig males.

This method of backcrossing with the Anfällig colony, always followed by a parathion selection (100 ppm) of the adult females was continued to the G_5.

It may be assumed that all surviving individuals in the G_5 were heterozygote to the factor A, as always a-males were used at every backcross. It must, however, be possible to make homozygotes by selection and inbreeding. To this effect all females of the G_6 and G_7 were 'screened' by placing them in cages on plants treated with parathion (100 ppm) before rearing an egg-wave.

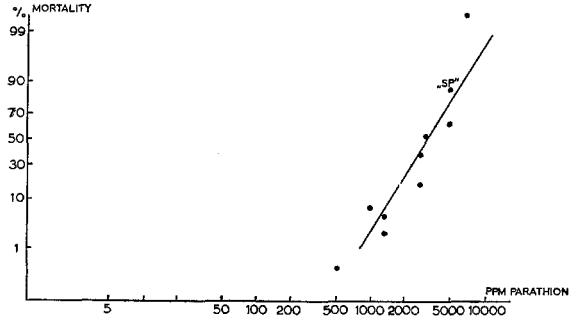

FIG. 12. Susceptibility of strain SP to parathion (3 experiments).

After the selection in the G_7, eleven adult females were placed each separately on a bean leaf. Three further generations of each of the eleven monocultures were produced by inbreeding. The females in the G_{10} were tested for parathion resistance, by placing them in cages on parathion (100 ppm) treated plants. In eight of the eleven inbreeding lines formed the material with which a new strain, called SP was started. This strain was reared in isolation in a thermostat controlled cabinet without contact with toxicants.

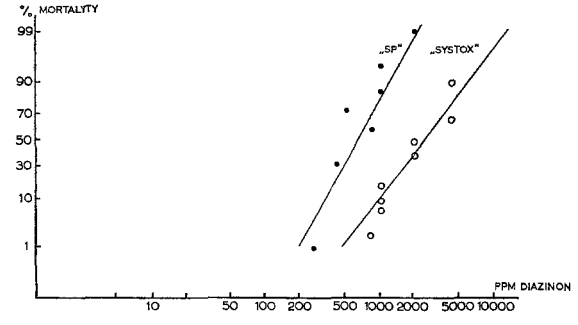

FIG. 13. Susceptibility of strains SP and Systox to diazinon (3 experiments).

Result. Figure 12 shows the susceptibility of strain SP to parathion. The LD_{50} of SP is 3311 ppm. As has been explained before (pag. 16), it is impossible to determine the LD_{50} of strain Systox with the chosen method.

The fact, however, that SP is clearly more susceptible to parathion than the Systox-strain, was the incentive to examine the level of resistance of both strains for another OP-compound. Diazinon gave the possibility to use a dosage range, also comprising the LD_{50} of the Systox strain (fig. 13).

The susceptibility to diazinon of strain SP is appreciably greater than that of strain Systox ($p. < 0.001$), in other terms the resistance has partly been reduced. These data indicate that in addition to factor A other factors are active, which may determine the resistance level.

5.6. DISCUSSION

The results of the tests with OP-resistant and -susceptible mites previously described, all lead to the conclusion that one gene is responsible for the major part of the resistance. The F_1 of the R $\times$ S and S $\times$ R crossings show that the resistance is dominant and that it is transmitted by both sexes. The F_2 of the S $\times$ R crossings consists of two types of females, viz. resistant and non-resistant in a 1:1 ratio, as might be expected in an arrhenotokous species transmitting a single factor. Though resistance is a quantitative characteristic, which seriously impedes a reliable analysis of phaenotypes, in a series of discriminating parathion doses between 50 and 1000 ppm, a numerical relation of the two types (resistant and non-resistant) was clearly visible.

It was not possible to break down the parathion resistance by means of backcrossing in combination with low pressure selection, which is in agreement with the fact that a major gene exists. In an alternative situation, e.g. resistance determined by cumulative polymery, the back-crossing system would lead to segregation of factors and as a consequence the SP strain would show a lower resistance level than has been found.

The lower level of resistance of strain SP as compared with strain Systox, leads to the assumption that in addition to the resistance gene indicated by A, other hereditary factors are involved. This lower level is most evident, if the diazinon resistance of SP and Systox are compared. A genetic analysis of these factors may prove extremely difficult, because their effect is very small and a genetic segregation of these factors will be hard to demonstrate, owing to the fact that the regression lines of SP and Systox are overlapping to a large extend (see fig. 13). The mode of selecting strain Systox from the Anfällig colony may explain the difference between Systox and SP. Systox was selected from the Anfällig colony with an rather low initial selection pressure of 60% mortality, and the selection pressure was increased in the following generations. This mode of selection may conceivably result in less specific genes being also transmitted which can play a prominent part in the Systox strain. Moreover, the Systox strain was occasionally treated with demeton after the selection proper, to maintain a high resistance level. It is quite conceivable, that this resulted in selection for other characteristics, called by HOSKINS & GORDON (1956) 'vigor tolerance'.

The results of the present investigation are in accordance with those of TAYLOR

& SMITH (1955) and ANDRES & PROUT (1960); however, they are not in agreement with those of DITTRICH (1961).

When backcrossing *T. urticae*, DITTRICH was able to break down resistance to a S level in three generations. This raises the question, whether any other factors may be involved. In resistant strains DITTRICH finds a relatively rapid reversion due to decreased egg production, higher mortality, retarded development, diminished resistance to food shortage, reduced sucking activity and a shift in the sex ratio in favour of the male.

We have been culturing R mites for many years without the use of insecticides, but there has never been a sign of reversion to susceptibility. In this connection an observation may be reported relating to an attempt to raise the OP-resistance in the Baardse colony to a maximum level, a 'superresistance'. After some parathion selections with 5000 ppm and over, the surviving mites produced few eggs and took significantly less food than before the selections. Moreover, a great part of the eggs was sterile, and the males were in excess of the females. For this reason the selections were discontinued, but the factors of decreased viability just described remained during some generations.

DITTRICH started from an R strain which was kept at the highest possible R level by repeated selections with a high dose of demeton. His strain may, therefore, more or less be compared with our strain Baardse after the selections with 5000 ppm parathion. It does not appear conjectural to assume that in both strains less specific genes have occurred in a relatively high frequency besides a specific resistance.

Also the difference found between our strains SP and Systox may support this view, because here it was shown that other factors may be present in Systox besides the specific major factor A. By inter-action with the major factor for resistance, these genes may contribute to a high resistance level; they may, however, at the same time be harmful for the entire viability of the population. It should be borne in mind that such a super R population is less suitable for a genetic analysis of the resistance by means of crossing experiments, due to the complicated background-variability which will interfere with the results.

The rapidly acquired parathion resistance of spider mites in Aalsmeer glasshouses may be readily explained by monofactorial resistance. Elsewhere too, e.g. in the U.S.A. (SMITH, 1959) only a few applications of OP-compounds were found to result in considerable resistance. ANDRES & PROUT (1960) describe, how by a single parathion treatment a population of *T. pacificus* was partitioned into susceptible and resistant classes.

An important aspect of such a rapid increase of resistant individuals in a population under selection pressure, is the arrhenotoky of Tetranychids. There are only two genotypes of males, being *A* and *a*. Normally, as is the case in *Musca*, three genotypes can occur: *AA*, *Aa* and *aa*. The presence of males being heterozygous for the *A* factor, resistance has consequently a less rapid development. A simple calculation of the increase of the frequency of a dominant gene (*A*) at selection against the recessive *a* gene can illustrate this.

Starting from a very large *Musca* population, containing the *A* gene at a very low frequency, so that only heterozygotes for this character occur in the population, only the genotype *Aa* will survive after a treatment with a discriminating

dose of a toxicant. When giving the future generations the same selection pressure (coefficient of selection s = 1.00), the offspring G_1 (first generation) will consist of the genotypes *AA*, *Aa* and *aa*, in the relation 1:2:1 (assuming no differential viability between the genotypes). The frequency of the *A* gene has thus increased from 'very rare' to 0.5.

In the G_1 the genotypes *aa* will disappear owing to the continually prevailing selection pressure, before they become fertile. In case the fertility of *AA* and *Aa* is equal and at random mating, a G_2 will be obtained with the three genotypes *AA*, *Aa* and *aa*, in a relation of 4:4:1, so that the frequency of the *A* gene is 0.67. A same selection in the G_2 causes a genotype relation in the G_3 of *AA:Aa:aa* = 9:6:1, amounting to an *A* gene frequency of 0.75.

The values of frequency of *A* in the different generations in the chosen example thus follow the sequence: $G_1 = 1/2$; $G_2 = 2/3$; $G_3 = 3/4$; $G_4 = 4/5$, every factor being given in the equation:

$$p_t = \frac{t}{t+1} \qquad t = 1, 2, 3 \ldots$$

in which p_t is the frequency of the *A* gene and t the number of generations.

Considering at present a similar population of *Tetranychus urticae*, also with a very rare A gene so that only *Aa* females occur, and also some males are in the happy possession of the *A* gene, a G_1 will occur after selection (s = 1.00) showing equal ratio of females of the genotypes *AA* and *Aa*, the males appearing in two genotypes as well (*A* and *a*), also in a 1:1 ratio. The frequency of the *A* gene of the spider females in the G_1 is therefore 0.75; and of the males 0.50.

If the *a* males are excluded from reproduction we shall find in the G_2: an *A* gene frequency of the females of 7/8, whereas the relation of the *A* and *a* males will be 3:1. After t generations the frequency of the *A* gene of the females will be:

$$p_t = \frac{2^{t+1} - 1}{2^{t+1}} \qquad t = 1, 2, 3 \ldots$$

In the above theoretical and simplified example we assumed random mating (panmixia), so that any individual has an equal chance of mating with any other individual in the population. These conditions are not fulfilled in a *T. urticae* population, as the phenomenon of sibmating is not rare in spider mites.

In *T. urticae* a fertilized female migrates to a fresh leaf, on which a number of haploid and diploid eggs are deposited closely together. On this spot males and females will develop from those eggs respectively. Copulation between brothers and sisters will occur frequently, because as a rule only *after* copulation the tendency to migration will appear. In a population of spider mites local accumulations of rare genes may spring up.

It is clear that also sibmating will positively influence the rate of increase in resistance, as R genes with a low frequency can become homozygous in a population by sibmating.

6. DIAPAUSE IN CONNECTION WITH OP-RESISTANCE

6.1. INTRODUCTION

From an extensive survey of the occurrence and distribution of OP-resistance in spider mites, made at Aalsmeer in 1958, it appeared that resistance was limited to glasshouses. Spider mite colonies, found on weeds and border plants near the glasshouses were normally susceptible to parathion and other OP-compounds (HELLE, 1959).

At first sight this phenomenon is exactly what should be expected. It is very rare that spider mites are chemically controlled at Aalsmeer outside the glasshouses. Selection pressure is, therefore, not present outdoors and there seems to be no reason for the mites to develop resistance. There is, however, a very strong selection pressure acting in the glasshouses and resistant mites are actually found there in great numbers. It is inevitable that resistant individuals will migrate from the glasshouses and start to develop outdoor colonies on plants close by. Such migrations e.g. of *Tetranychus cinnabarinus* from carnation houses have been actually observed (VAN DE BUND & HELLE, 1960).

Yet it was noted that only S mites were found outside glasshouses. Apparently the resistant escapees were unable to establish themselves outside the glasshouse. The assumption was made that a difference in hibernation possibilities between S and R mites might be the cause of this fact, and it was supported by a number of trials to let resistant mites hibernate outside.

In the late summer of 1959 and 1960 bushes of *Buddleya davidii* (Franch) were infected with OP-resistant spider mite colonies from glasshouses. Another *Buddleya* bush was infected with S mites. The infections were all completely successful. *Buddleya* is a hostplant which is very suitable for hibernation, as the bark of stem and branches provides many crevices and the pitch near old prunings also offers suitable cover for hibernation. In winter often thousands of diapause forms of *T. urticae* can be found in such refuges. Moreover, *Buddleya* shoots early in spring and the diapause mites become active on the leaves already in April, where they find enough food to start colonies. However, in the spring of 1960 and 1961 no resistant mites could be found on the infected bushes. On the bush infected with S mites, mites susceptible to parathion were found in both years. This remarkable observation was the direct reason for a more exhaustive investigation of the hibernation of S and R mites.

The first object of study was to see whether there are differences between R and S mites with regard to conditions of light and temperature prevailing during development which may lead to diapause in adult females.

It is well known that the critical photoperiod may vary in many species of Arthropods which have a facultative diapause (LEES, 1956). DANILIEVSKI (1957) showed that the differences of the critical periods found in various populations of *Acronycta rumicis* (L.) were the result of an adaptation to the conditions of the latitude at which this moth lives. At high latitudes the days are longer, but winter generally sets in earlier. Consequently at high latitudes diapause will start at a greater day-length than at lower latitudes.

There are indications that similar photoperiodical races of spider mites can be distinguished. The critical photoperiod of *T. urticae* from Leningrad (60 °N) was 16 hours 40 minutes (BONDARENKO, 1950, 1958) and of those from Cam-

bridge (52 °N) 13–14 hours (LEES, 1953a). This may lead to the conclusion that Leningrad mites enter diapause at an earlier date than Cambridge mites. Further investigations of BONDARENKO & KUAN (1958) on diapause in spider mite populations from various latitudes (Leningrad, Krasnodar, Tiflis and Tashkent) led to the conclusion that the critical photoperiod increases by one hour per three degrees of latitude.

A geographical variability in critical photoperiod could be taken into consideration, when comparing S and R populations. Glasshouse populations often originate from cultivated crops, possibly imported fromc ountries of another latitude and consequently a different diapause pattern.

For a tentative exploration of the diapause of S and R mites, a number of spider mite colonies were collected from glasshouses, where OP-resistance was known to have existed for years. The diapause of susceptible strains, all of which, with the exception of Anfällig, were collected outside the glasshouses, was used for comparison.

6.2. METHOD

Thirty adult females were placed on a young bean leaf in a Petri dish. Eight hours afterwards the females were removed, and only eggs of the same age (average 51 per dish) were left behind on the leaf. For three consecutive days the Petri dishes with the eggs were kept in a glass box at 25 °C under continuous fluorescent light. On the fourth day the Petri dishes with the eggs were placed in the containers of a serial thermostate. This serial thermostate consisted of five containers of 50 × 50 × 50 cm each. Each container could be independently adjusted to temperatures ranging from 5 to 35 °C with intervals of one degree, and was illuminated by fluorescent lamps. On the bottom the light intensity was 1200 Lux. The duration of the light and dark periods was controlled by pinwheel timing devices.

The air humidity in the containers could not be regulated exactly, but with the aid of silica-gel it was kept between 65 and 90%.

The mites completed their development in the containers, and all stages from larvae to adults were therefore subjected to a definite photoperiod and temperature.

When the mites had reached the adult stage, they were kept in the container for another week. This was important, because the orange pigmentation which is typical for diapause, often appears some time after the mites have reached the adult stage.

In checking the percentage of diapause females they were distinguished from active females according to colour, irrespective of the question whether reversal to the active form was possible or not. In many cases the mites were found to be only partly orange coloured. Such individuals were also enlisted in the diapause group.

For each test at least one Petri dish was used for replication. The diapause percentages relate to at least 80 females.

6.3. OCCURRENCE OF DIAPAUSE IN VARIOUS S AND R COLONIES

1. *General remarks.* Four susceptible colonies of mites were used, viz. Chelidonium and Anfällig, referred to before, an outdoor colony from *Buddleya*

davidii from Aalsmeer and a colony from Basel (Switzerland) found on *Phaseolus vulgaris*. For the duration of the experiment the latter two colonies were kept in a non-conditioned room, and isolated by means of a water barrier.

Besides, strains of spider mites were collected at Aalsmeer and in the Belgian grape district south of Brussels. In these colonies the susceptibility for parathion was tested and nine were found to be resistant. The resistant strains were kept in culture and used for the experiments.

Four different temperatures were used, viz. 15, 18, 20 and 22 °C and two periods of light, viz. 4 and 12 hours. Intentionally the combinations were chosen in order to provide both weak (22 °C, 12 hours of light) and very strong diapause conditions (15 °C, 12 hours of light). Table 6 shows the diapause percentages at various combinations of photoperiod and temperature.

TABLE 6. Diapause percentages of four S-colonies and nine-R-colonies of different origin.

Strain	Original hostplant	Country	Percentage diapause females				
			15°[1]) 4h	15° 12h	18° 4h	20° 12h	22° 12h
Chelidonium	Chelidonium	Netherlands	98	100	71	99	52
Gasser	Phaseolus	Switzerland	100	100	98	100	60
Buddleya	Buddleya	Netherlands	98	100	90	100	38
Anfällig	Phaseolus	Germany	100	100	92	100	84
II	Rosa	Netherlands	20	75	6	0	0
Baardse	Rosa	Netherlands	0	2	0	0	0
Terlouw	Rosa	Netherlands	0	0	0	0	0
Markman	Rosa	Netherlands	96	100	50	72	20
Adriaenssens	Vitis	Belgium	96	100	46	8	0
S 108	Vitis	Belgium	94	98	22	3	0
S 109	Vitis	Belgium	98	98	33	8	6
Herenthals	Vitis	Belgium	5	10	2	0	0
Duphar	Vitis	Belgium	28	95	55	20	0

[1]) Degree of temperature in centigrades and amount of time in hours.

The data of the S strains are roughly in accordance with the values found by LEES (1953a). It was expected that the percentage of diapausing mites of the Gasser-colony from Basel at 22 °C and a photoperiod of 15 hours would be inferior to the percentages of diapausing mites from Buddleya and Chelidonium, because Aalsmeer (52 °N) has a higher latitude than Basel (47 °30'N). However; rather the opposite has been found. In this connection it should be borne in mind that it may be doubted whether the Basel colony is really indigenous, since it has been collected from a cultured crop.

The percentages of diapausing mites of the R colonies are remarkable. Not a single combination of temperature and photoperiod in the Baardse, Terlouw and Herenthals colonies resulted in an appreciable percentage of diapause. As a rule only active forms developed, i.e. those which immediately after emergence started sucking and laying eggs. A striking feature of these colonies was the increased mortality, of which, however, no exact observation has been made.

Four R colonies (S 108, S 109, II and Duphar) also show a smaller percentage of diapausing animals as compared with S colonies, although the differences are not so marked as in the former three. In two R colonies (Markman and Adriaenssens) a temperature of 15 °C and a photoperiod of 12 hours, inducing

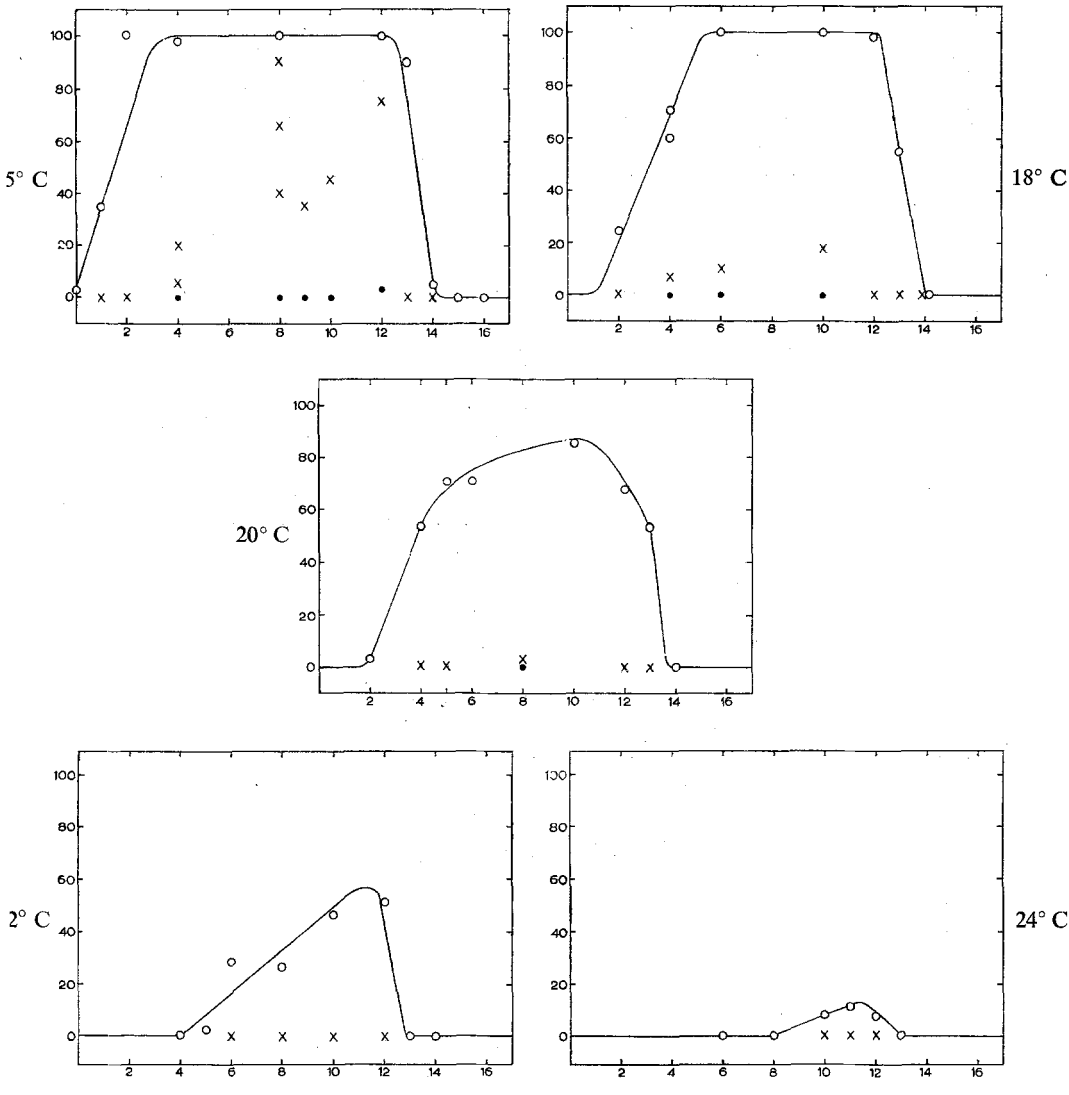

FIG. 14. Diapause percentage at various temperatures (15, 18, 20, 22 and 24 °C) and photoperiods. Horizontal: photoperiod in hours per 24 h; vertical: percentage of diapause females.
ooo = Chelidonium colony
xxx = II colony
. . . = Baardse colony

100% diapause in the S colonies, even leads to 100% diapause. At higher temperatures (20 °C) there is the same tendency of high percentages of diapause in the S colonies, and of low percentages in the R colonies. The exception is the Markman colony, with diapause percentages approaching those of the S colonies.

Briefly summarized, the R and S spider mite colonies react differently to the regimen of photoperiod and temperature, the S mites providing more diapause forms. This phenomenon was also found bij SABA (1961) at a regimen of a photoperiod of 9 hours and a temperature of 13° C. Our various R colonies, however, show marked quantitative differences in this respect, some colonies showing no diapause-forms at all, others reacting with an intermediate percentage.

2. *Diapause of colonies II and Baardse.* Two R colonies, viz. II and Baardse from localities in the Netherlands, were subjected to a greater number of combinations of photoperiod and temperature. For purpose of comparison the Chelidonium colony (S) was tested at the same temperature and length of day. The diapause pattern at various temperatures is shown in fig. 14.

The experiments in absolute darkness (0 hours of light) could not be done in the same way as the others, as the bean leaves in the Petri dish then quickly turn yellow. The condition of the hostplant is an important factor in the occurrence of diapause, as was demonstrated by LEES (1950, 1953a). In this experiment, therefore, the mites were transferred to fresh leaves.

The Chelidonium colony will enter into diapause at 15 °C and a photoperiod of 0–14 hours 10 minutes. When the temperature is raised, the critical photoperiod will shorten, at 18 °C it varies between 1 and 14 hours, at 20 °C between 1 hour 40 minutes and 13 hours 40 minutes, at 22 °C between 4 hours 30 minutes and 13 hours, and at 24 °C between 8 hours 30 minutes and 12 hours 30 minutes. At temperatures of 25 °C and over, no diapause could be induced. This is contrary to the findings of LEES (1953a) and BONDARENKO & KUAN (1958), who were able to induce diapause at these temperatures. The difference in hostplant may be an explanation of this discrepancy.

When producing the raising lines of fig. 14 (18 °C), we find a point of intersection. From this point the photoperiod can be calculated, which will induce the maximum diapause percentage. The strongest photoperiodic stimulus is found between 10 and 11 hours light a day.

At temperatures of over 20 °C the R colonies II and Baardse show no diapause at all, whereas at 20 °C only a few mites of the II colony enter into diapause.

Considerably more diapause mites are found in II at 15 °C than at higher temperatures. However, the results of the tests were not very reproduceable. A test with mites of the II colony at 15 °C and 8 hours of daylength was repeated twice, giving the following results: 91%, 40% and 66%. It is most likely that this is caused by a great heterogeneity as to this characteristic.

The Baardse colony showed scarcely any diapause at all. In the experiment at 15 °C and a photoperiod of 12 hours, three mites were observed missing the orange colour, typical for diapause: the mites had a brownish colour. They were isolated and behaved like diapause individuals in that they did not feed nor did they lay eggs.

6.4. DISCUSSION OF THE PROBLEM

The fact that R colonies of *T. urticae* will not or only partly enter diapause, has remarkable consequences. With reference to diapause the R populations react as populations of a lower latitude, as described by BONDARENKO & KUAN

(1958) for *T. urticae* from Tiflis and Tashkent. At a photoperiod of 11 hours at most 90% of these populations showed diapause, while longer photoperiods resulted in almost total absence of diapause. In northern climates, where winter sets in earlier, populations with such a restricted diapause pattern will not be able to live in the field. Either they will not enter diapause, or the onset of diapause will come too late. This would provide a simple explanation of the hibernation difficulties of resistant spider mite populations at Aalsmeer.

The question may be raised, whether the relation between decreased diapause and OP-resistance is causal or just a matter of coincidence. The tested R populations were all taken from glasshouses. However, glasshouses provide abnormal environments: in late summer and autumn the daylight will shorten in accordance with the latitude, but temperatures will generally be kept many degrees above those outside the glasshouses. In indigenous spider mite populations (viz. those able to maintain themselves in glasshouses for many generations) this may have its repercussions on their diapause pattern. Thus mites entering diapause early in the year will be at a disadvantage. In a glasshouse, heated in winter, with temperatures not below 15 °C, diapause will be fatal in most cases, as the chilling period mostly required for the reactivation will not be effectuated and death of the majority of the diapause mites will occur (LEES, 1953a). Even the progeny of diapausing mites which come to reversal can no more successfully compete with the active forms. So the population will be subjected to a heavy selection pressure, and the active mites will form the greatest part in the growth of the colony. Conceivably in the course of time spider mite strains will have developed which are no longer reactive to any photoperiod.

According to this theory, OP-resistance and non-diapause would only correspond more or less by accident: the chemical control caused another selection pressure on the non-diapause mites in the glasshouses, resulting in OP-resistance.

The opposite supposition has also been made, considering the OP-resistance as the primary phenomenon. Owing to the development of resistant populations in the glasshouses for the last ten years, it may be assumed that a sort of indigenous population came into existence. Such populations prospered year after year in spite of chemical control and moreover possessed sufficient adaptability to produce non-diapause mites. In this case too, not a causal relation between resistance and non-diapause exists, but an accidental connection between two adaptations, one to the glasshouse climate and the other to the chemical control.

In order to investigate to what extent the relation is accidental or causal, a series of selections were effected in the laboratory on a S colony with a normal diapause pattern. In the first place an effort was made to select for non-diapause, to find out if changes in the diapause pattern are attended by changes in parathion susceptibility. The opposite was also tried, i.e. if parathion selection of a S colony may lead to a decreased diapause in the laboratory.

6.5. SELECTION FOR NON-DIAPAUSE IN THE LABORATORY

These selections are based on the systematic removal of diapausing females from further reproduction.

The experiment was based on an egg-wave of the S colony Chelidonium, over

a thousand eggs, distributed over 14 leaves in Petri dishes. For three days the eggs were kept at a temperature of 25 °C. Just before hatching they were transferred to a container with a temperature of 20 °C and a photoperiod of 5 hours, thus inducing diapause in 70% of the females. After having reached the adult stage, the active females were transferred to fresh leaves in Petri dishes, to obtain a new egg-wave. The dishes were kept at 25 °C and the females were removed after two days. The new egg-wave too was kept at this temperature until new larvae hatched. In turn they were transferred to a container for further development.

After five selections for non-diapause a culture of the G_5 was made to check the susceptibility to parathion. This was repeated with the G_7.

TABLE 7. Selection procedure on non-diapause with the Chelidonium colony.

Generation	Selection	Total females	% diapause females obtained
G_0	20° 5h [1])	973	74
G_1	20° 5h	312	81
G_2	20° 5h	342	73
G_3	20° 5h	166	19
G_4	20° 8h	214	8
G_5	18°12h	170	34
G_6	18°12h	122	7

[1]) Degree of temperature in centigrades and amount of time in hours

The selections are shown in table 7. Only after the third selection a clear decrease in the percentage of diapausing forms occurred, and another light-temperature combination had to be used. It is remarkable that all combinations subsequently made of short day-length and temperature were chosen too prudently, as at 20 °C/8 hours and 18 °C/12 hours, only a small percentage of the females entered diapause. After the G_7 the selections were discontinued and from the remaining active specimens, colonies were reared designated as strains Chel-ND_5 and Chel-ND_7 from the G_5 and the G_7 respectively.

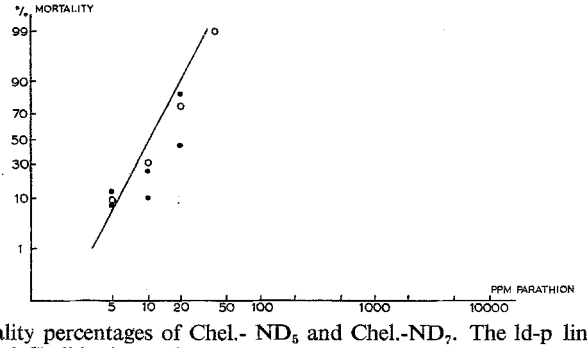

FIG. 15. Mortality percentages of Chel.- ND_5 and Chel.-ND_7. The ld-p line represents the original Chelidonium colony.
ooo = Chel-ND_5
... = Chel-ND_7

Some tests were made on strain Chel-ND_7 to give an impression of the diapause at 15 °C. The percentages amounted to 79 and 92, with photoperiods of 4 and 11 hours respectively. At 18 °C and the same photoperiods, percentages of 0 and 4 respectively were found. Though at 15 °C still considerable, the diapause of Chel-ND_7 has practically disappeared at higher temperatures.

The susceptibility to parathion of Chel-ND_5 and Chel-ND_7 is shown in fig. 15. As the calculated percentages do not deviate from the ld-p line of Chelidonium, it may be presumed that the parathion susceptibility has not changed appreciably.

6.6. DIAPAUSE IN LABORATORY STRAINS SELECTED WITH OP-COMPOUNDS

The parathion selections were made with the Chelidonium colony. To this effect a representative part of this colony was reared in a thermostate controlled cabinet under continuous lighting at 25 °C. When new hostplants were placed in the cabinet, they were infected by means of pieces of leaf infested with eggs, the difference in age varying from 0 to 4 days. When the greater part of the mites had reached the deutonymphal stage, they were treated with ethyl-parathion in a concentration of 80 ppm. This dosage caused an average mortality of 90%. This rather high selection pressure made it necessary to treat every second generation, in order to permit the population to return to its previous level.

During eighteen generations, selections were made ten times with an 80 ppm dosage of ethyl-parathion, without any significant change in the parathion susceptibility of the selection strain. It may be assumed that no factors of resistance were present in our Chelidonium colony. Other investigations e.g. by WATSON & NAEGELE (1960) have shown resistance to develop after a few selections, both under high and low pressure. ANDRES & PROUT (1960) obtained resistance in *Tetranychus pacificus* after one single selection.

The failure of the Chelidonium colony selection necessitated the choice of other material. The strain Systox was obtained from Professor Unterstenhöfer, Leverkusen. This OP-resistant strain was selected from the Anfällig colony by means of a small number of demeton treatments. The selections were made under laboratory circumstances with long day-length conditions. This strain met the required standard, and the diapause was thoroughly tested. The Anfällig colony was also tried for purpose of comparison. Fig. 16 shows the percentages of diapause at 15, 18, 20, 22 and 24 °C and various photoperiods. At a temperature of 15 °C diapause will occur at any photoperiod under 14 hours 10 minutes per day.

The diapause percentages of the R strain Systox have markedly decreased. At temperatures of over 18 °C diapause is practically non-existent. At 18 °C and a photoperiod of 10 hours only 51% of the Systox females enter diapause, as against 100% of the Anfällig females. At 15 °C diapause in the Systox strain is more prevalent; however, 100% is not reached for any day-length.

This result seems strongly in favour of a genetic association between OP-resistance and decrease of diapause. The selections with demeton resulted in OP-resistance as well as in a decrease of diapause. The question may now be asked, what relation there may be to the major factor A, shown to be present in strain Systox.

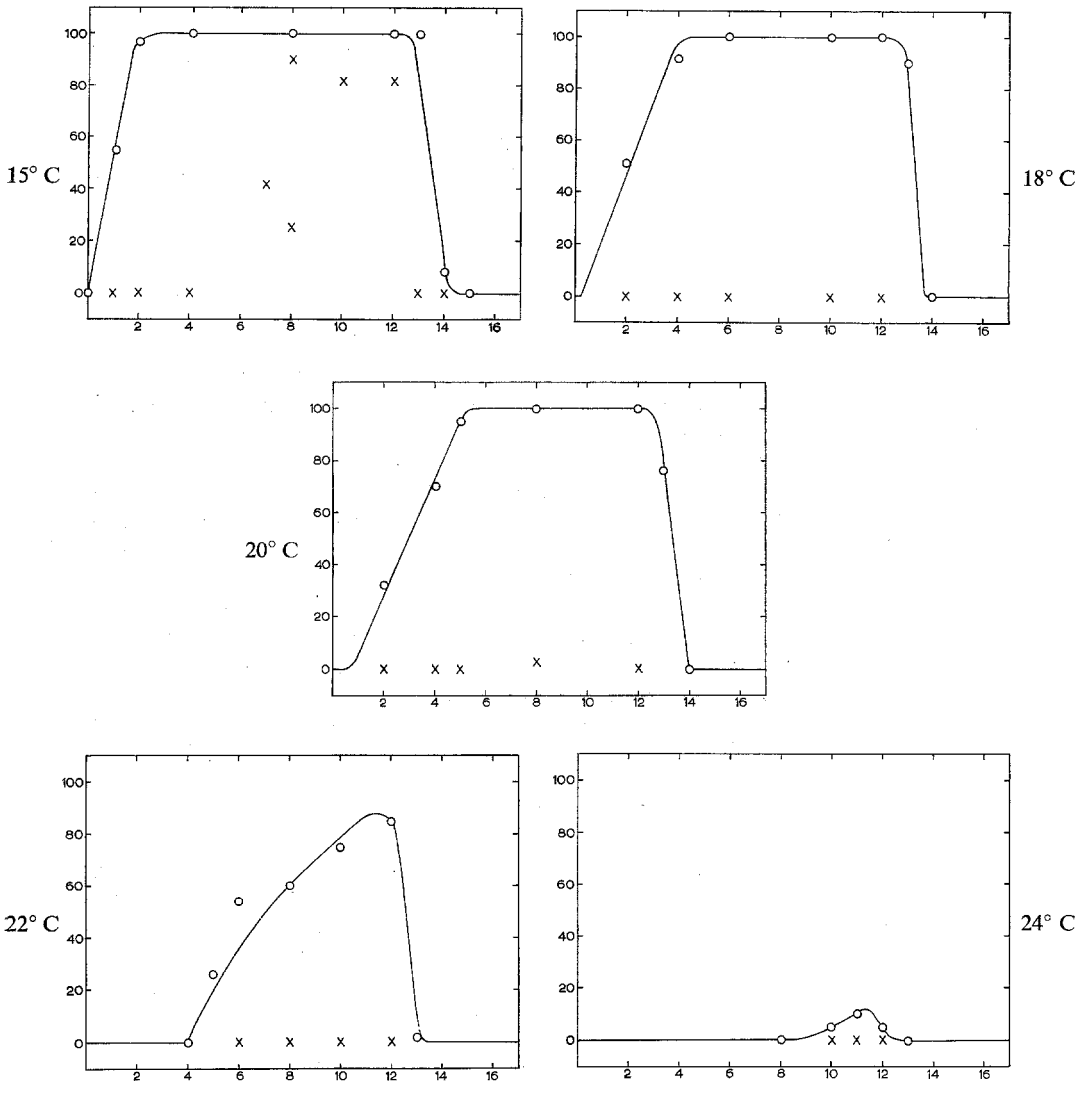

FIG. 16. Diapause percentages at various temperatures and photoperiods.
ooo = Anfällig colony
xxx = Systox strain

6.7. DIAPAUSE IN STRAIN SP

Any selection for a desired character x involves inevitably the selection of other hereditary factors, some of which may be more or less closely linked with character x. Others, however, may have been accidentally passed on, and become more apparent in the selection strain.

To a large extent such characteristics, however, will have been reduced to the

original frequency in the SP strain (see page 175 and fig. 11). This strain was obtained by the procedure of repeated backcrossings of the hybrid of Anfällig × Systox with Anfällig, while selecting for parathion resistance, and here the resistance-factor A has been isolated from most of the less specific and less important resistance factors and assimilated in the Anfällig genome. Accidental characteristics must have been largely replaced by alleles of the Anfällig genome during the backcrossing.

For this reason strain SP appears very suitable to investigate what relation may exist, if any, between the major factor A and diapause.

In table 8 the diapause percentages of this strain are compared with those of Anfällig and Systox at various combinations of temperature and photoperiod.

TABLE 8. Comparison between diapause percentages of the Anfällig colony, strain Systox and strain SP.

	15°12h[1])	15°4h	18°4h	22°6h	22°8h	22°10h	22°12h	24°12h
SP	97	94	98	0	10	65	80	0
Systox	82	0	0	0	0	0	0	0
Anfällig	100	100	92	55	61	75	84	4

[1]) Degree of temperature in centigrades and amount of time in hours.

The diapause percentages of SP make it plausible that there is no question of pleiotropy; the diapause has largely returned. At a temperature of 22 °C, when strain Systox will not enter diapause at any photoperiod, a considerable percentage of diapause was induced in the females of SP. The percentages approach those of the Anfällig colony.

6.8. DISCUSSION OF RESULTS

The selections for non-diapause of the Chelidonium colony, resulting in a strongly reduced number of diapause forms at 20 °C, answer the question why *T. urticae* is capable of satisfactory adaptation to the peculiar environment of a glasshouse, as regards diapause.

Glasshouses where plants are grown with a short cultivation period do not permit spider mites to maintain themselves for years. Rose houses, however, present quite a different situation. Roses are cultivated during a long period of time (average 7 years), which is an important factor favouring the adaptation to hibernation in glasshouses, though environments in winter may be subject to variations.

Some roses, e.g. *Rosa* hybr. 'Parel van Aalsmeer', 'Better Times', 'Briarcliff' and 'G. I. Joe', require heating throughout winter. In such glasshouses the average temperature in winter is 15 °C and also at that time roses have still their foliage. In autumn *T. urticae* will be trapped in diapause: the short day induces diapause, but the required chilling period will be lacking. In this type of glasshouse, spider mite populations in which diapause seldom occurs, which is characteristic to the Baardse- and the Terlouw colony, are adaptated best to the circumstances.

'Roselandia', 'Baccara', 'Mad. Ofman' and 'Geheimrat Duisberg' are roses,

which require another type of glasshouse culture. These roses have a short or long resting period in winter, when they lose their leaves, while the temperature in the glasshouse may fall to freezing point. Time and duration of the resting period, dependent on economic factors and the nurseryman's insight, are far from being constant. Such a period may last for four weeks, but it may be extended to six weeks. It may commence in November, but considerably later too. Spider mites with a fixed diapause pattern will do badly in these glasshouses. Populations with a larger adaptation potential are the ones which will be able to maintain themselves.

The Chelidonium colony experiments justify the supposition that this must be the case. The rapid increase of non-diapause forms after a small number of selections leads to the assumption that such adaptations have occurred in glasshouses. This is in accordance with the grower's reports, that damage was done by active forms in October, November and December, even before 1948, when parathion was not yet used. The results of the selections of the Chelidonium colony may lead to the assumption that the changes in the diapause pattern of *T. urticae* populations in glasshouses are an adaptation response to the special environments. The ld-p line of strain Chel-ND_7 proves that a narrower diapause pattern will not cause an appreciable change of the parathion susceptibility.

From the remarkable relation between the reduce ddiapause and the OP-resistance in the Systox strain it may be surmised that changes in diapause could be effected directly or indirectly by resistance factors. What factors are involved is still a problem to be solved. The return of diapause in the SP strain excludes the possibility of pleiotropy of the major gene of resistance. It is conceivable too, that other and less specific resistance factors may be responsible for the reduction in diapause.

7. CONCLUSIONS

1. A single hereditary factor can cause a considerable resistance to parathion in *T. urticae*. Other factors in addition to this major factor may increase the resistance level.
2. Resistance is dominantly transmitted by both sexes.
3. The reduction in the percentage of diapause forms of parathion resistant strains of *T. urticae*, as compared to susceptible strains, is not due to pleiotropy of this major factor.
4. *T. urticae* possesses sufficiently large adaptation potential to maintain itself in glasshouses during wintertime. The resulting changes in diapause pattern do not necessarily lead to changes in the susceptibility to parathion.

SUMMARY

This publication deals in the first place with an investigation into the genetics of resistance to organo-phosphorus compounds in the two spotted spider mite (*Tetranychus urticae* Koch).

These experiments were carried out with two mite colonies, which were normal-

ly susceptible to parathion, and two other colonies which proved to be highly resistant to this compound. The colonies were bred in constant temperature boxes, specially adapted to this purpose, whereas provisions were made to isolate the colonies carefully. The susceptibility of these colonies to parathion was determined by spraying beanplants with a range of doses of parathion, after which a fixed number of females, of a definite age, were transferred to the beanplants. The differences in susceptibility were very large: the LD_{50} of the susceptible populations was about 11 ppm., whereas the LD_{50} of the resistant colonies remained above 3000 ppm. (fig. 3 and 4).

Crossings between susceptible (= S) and resistant (= R) individuals resulted into a resistant progeny. As these crossings were made reciprocally, the conclusion was drawn that resistance is transmitted by both sexes and has a dominant character (fig. 5 and 6).

An analysis of gametes was obtained by backcrossing the hybrid (S × R) × S. It was remarkable that various parathion concentrations within the range of 50–1000 ppm always produced a mortality of about 50% (fig. 9 and fig. 10). This indicates the presence of two categories, viz. R and S mites in a ratio of 1:1, which can be explained by assuming the existence of a major gene of resistance.

The next step was examining the level of resistance for which this major gene is responsible, as the first backcross was not conclusive at that point. To this purpose a number of backcrossings was brought about, in combination with selections of parathion (fig. 11). This method of crossing enables the isolation of the major gene. Repeated backcrossings of the F_1 of R × S with S males will substitute alleles from the S strain for minor factors of the resistance complex. Due to the low selection pressure (100 ppm parathion) the more important R factors will maintain their position, and will then be divested of possible modifying factors.

Originating from the R-strain 'Systox' a new strain 'SP' was formed, in which the resistance factor is found homozygous. The resistance-level of this strain SP proved to be four times as low as that of strain Systox (fig. 13). The conclusion was therefore drawn, that also other factors, probably modifiers, are operating in strain Systox.

In addition to a genetic analysis of resistance, investigations were made into the character of the relation between resistance and non-diapause in spider mites. In many resistant populations a regimen of photoperiod and temperature proved to provide a percentage of diapausing mites, that is considerably lower than in S-populations (table 6).

The R-strain Systox showed also considerably less diapause than the S-populations. In the strain SP, however, the percentage of diapausing mites proved to be normal, i.e. a percentage similar to that of S-populations (table 8). On account of this the occurrence of non-diapause cannot be explained from pleiotropy of the major gene of resistance. It is possible, however, that minor R-genes influence diapause.

Starting from a S-population with a normal diapause pattern, selections were carried out against diapause during six generations. The percentage of diapausing forms at a definite photoperiod and temperature, appeared to have

decreased after a few selections (table 7), however without any alteration in the susceptibility to parathion (fig. 15).

In hothouses, the quality of non-diapause has an outstanding positive selection value. On account of this, it is assumed that the diapause pattern of a spider mite population has been altered, and non-diapause must be considered to be an adaptation to the hothouse-climate.

As especially in hothouses, there is a selection pressure of insecticides on a spider mite population, it may be possible that the relation between resistance and non-diapause is based on a coexistence of two adaptations.

SAMENVATTING

Deze publicatie handelt in de eerste plaats over een onderzoek naar de genetische achtergrond van de resistentie voor bestrijdingsmiddelen op basis van organische fosforverbindingen bij de bonespintmijt (*Tetranychus urticae* KOCH).

Hierbij waren twee spintkolonies betrokken, die normaal gevoelig waren voor parathion, terwijl twee andere kolonies in hoge mate resistent waren voor dit middel. Deze kolonies werden in geconditionneerde ruimten en streng geïsoleerd van elkaar gekweekt.

De gevoeligheid voor parathion werd bepaald door boneplanten met een reeks doseringen parathion te bespuiten, waarna voor elke dosis een bekend aantal spintwijfjes van een bepaalde leeftijdsklasse op de boneplanten werd gebracht. Er bleken dan zeer grote verschillen te bestaan tussen de gevoelige (= G) en de resistente (= R) populaties. De LD_{50} van de G-kolonies was ongeveer 11 ppm parathion, terwijl de LD_{50} van de R kolonies boven de 3000 ppm lag (fig. 3 en 4).

Kruisingen tussen G en R mijten leverden een resistente nakomelingschap op, zowel bij G × R als bij de reciprook R × G. De resistentie is dominant en wordt via beide sexen overgeërfd (fig. 5 en 6).

De gametentest werd uitgevoerd door de hybride (G × R) terug te kruisen met G-mannetjes. Het bleek dat binnen het concentratiegebied van 50–1000 ppm parathion bij B_1-mijten een sterfte van ongeveer 50% optrad (fig. 9 en 10). Dit wijst op een samenstelling in de B_1 van twee groepen, nl. G en R mijten in een 1:1 verhouding. Deze uitkomsten kunnen slechts verklaard worden door aan te nemen dat aan de parathion-resistentie een 'major gene' ten grondslag ligt. Daar met de gametentest geen nauwkeurige informatie verkregen werd of naast dit ‚major gene' nog andere erfelijke factoren een rol spelen, werd met de R-stam ‚Systox' een serie terugkruisingen gedaan onder gelijktijdige selectie met parathion (100 ppm). De genetische consequentie van een dergelijke terugkruisingsprocedure is, dat het ‚major gene' grotendeels wordt ontdaan van minder specifieke factoren. Door terug te kruisen worden allelen uit het G-genoom geïntroduceerd, terwijl door de laag gekozen selectiedruk de belangrijke R-factoren gehandhaafd blijven (fig. 11).

Na de serie terugkruisingen werd door verdere selecties en door tussentijds opkweken van subpopulaties de stam ‚SP' opgebouwd, waarin de resistentiefactor homozygoot voorkomt.

Het resistentieniveau van SP bleek lager te liggen dan van de uitgangsstam Systox. Derhalve moet worden aangenomen dat andere genen (vermoedelijk

modifiers) in samenwerking met het ‚major gene' de resistentie aan stam ‚Systox' verlenen (fig. 13).

Naast een genetische analyse van de resistentie werd ook onderzoek gedaan naar de aard van de relatie tussen resistentie en non-diapauze bij spintmijten. Bij de meeste R-populaties ontstaan bij een samenspel van korte dag en lage temperatuur belangrijk minder diapauze vormen dan bij G-populaties (tabel 6). De vraag deed zich voor of deze relatie tussen resistentie en non-diapauze causaal dan wel accidenteel is.

De R-stam Systox, die door selecties met demeton uit een gevoelige stam was ontwikkeld, vertoonde belangrijk minder diapauze dan de uitgangspopulatie. Echter bleek bij stam SP weer normaal diapauze voor te komen (tabel 8), d.w.z. er trad een overeenkomstig percentage diapauze vormen op als bij de gevoelige populatie. Op grond hiervan kan het samengaan van resistentie en non-diapauze niet verklaard worden uit pleiotropie van het ‚major gene'. Wel is het mogelijk dat minder belangrijke R-genen de diapauze kunnen beïnvloeden.

Uitgaande van een G-populatie met een normaal diapauze patroon werd gedurende zes generaties op non-diapauze geselecteerd. Het percentage diapauze vormen dat bij een bepaalde daglengte en temperatuur optrad, bleek reeds na enkele generaties belangrijk verminderd te zijn (tabel 7), zonder dat echter de gevoeligheid voor parathion veranderde (fig. 15).

De eigenschap non-diapauze heeft in warme kassen een uitgesproken positieve selectiewaarde. Met het oog op het bovenstaande is het waarschijnlijk dat in stookkassen op non-diapauze geselecteerd wordt. Daar juist in kassen evenzo de selectiedruk van bestrijdingsmiddelen op een spintpopulatie rust, is het zeer wel mogelijk dat het samengaan van non-diapauze en resistentie verklaard kan worden uit adaptaties aan het kasmilieu, die onafhankelijk van elkaar zijn ontstaan.

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