

REDUCTION IN THE YIELD AND VIABILITY OF
CARROT SEED IN RELATION TO THE OCCURRENCE
OF THE PLANT BUG *LYGUS CAMPESTRIS* L.

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INTRODUCTION

The relatively poor germination of carrot seed is a weak spot in the production of good seed for both breeding and commercial purposes. This is known the world over and more or less accepted as a normal character of carrot seed. Also in the Netherlands it is a major problem of carrot seed production, to which a new one was added when in 1947 and 1949 serious reductions in the seed yield were experienced in various parts of the country.

This reduced yield resulted from a disorder, referred to in this paper as the "seedless umbels" disorder, which can be described as follows. The affected umbels are conspicuous, particularly in the seed-ripening period. At this stage normal umbels are fully expanded, their flat upper surfaces become light-coloured and woolly in appearance due to the long hairs on the numerous seeds. The seedless umbels, however, have a dark brown colour, the peduncles of the umbellets are standing close together with their tops turned inwards over the centre of the umbel.

Examination of affected plants by phytopathologists produced a negative result. Attempts to establish correlations with soil types and cultivation practices were also unsuccessful.

Therefore in 1951, at the special request of DR. C. RIETSEMA, Horticultural Advisory Officer at Hoorn, province of Noord-Holland, the investigation was taken over by the department of flower biology of our Institute.

PRELIMINARY OBSERVATIONS AND REVIEW OF LITERATURE

A preliminary investigation showed that in the ovaries of affected umbels production of gametes, pollination, fertilization and initial development of endosperm and embryo proceeded quite normally, but that later on a sudden check in the growth of the young fruits occurred, accompanied by abortion of endosperm and embryo (1).

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As bugs were sometimes found on carrot umbels collected in the field, and as injury resulting from the feeding of bugs seemed to us an acceptable explanation of the symptoms observed, we have concentrated our attention on the activity of these insects.

When in July 1953 our search for bugs was started we found adults and nymphs, sometimes in large numbers, on umbels in nearly every carrot field visited in Noord-Holland, as well as on carrot plants flowering in the experimental garden of the Institute at Wageningen. The majority of the collected animals proved to belong to the species *Lygus campestris* L. and only a few to the species *Anthocorus nemorum* L. Our sincere thanks are due to Mr. W. H. GRAVENSTEIN in Amsterdam for his valuable help in identifying the bugs collected in the field or used in our experiments.

FLEMION and her colleagues at the Boyce Thompson Institute for Plant Research have made a comprehensive study of the reasons for poor germination generally occurring in Umbelliferae. They found that the seeds from plants belonging to this family (including carrots) often contain a normal endosperm but no embryo, and that the low germination percentage was due to these embryoless seeds (2,3). Further investigations revealed that embryoless seeds were produced almost exclusively after plant bugs belonging to the genus *Lygus* had been feeding on the umbels (4). In this case the test plant was dill (*Anethum graveolens* L.) and the attacking insect the tarnished plant bug (*Lygus oblineatus* SAY.).

The results from these American investigations were of importance for the solution of our own problem. In the first place they showed that in Umbelliferous plants the feeding of *Lygus* bugs can result in embryo abortion. We had encountered numerous bugs of the species *Lygus campestris* on carrot plants in fields around Hoorn, the area where seedless umbels have been reported most frequently, and found that the check in fruit development of the affected umbels is correlated with abortion of the embryo. If the seedless umbels in the carrot are caused by *Lygus campestris* in the same manner as embryoless seeds result from feeding injuries of *Lygus oblineatus*, this would explain not only why in both cases there is no relation with heredity of the plants, soil condition, application of fertilizer or spacing of the plants (3), but also why there are such large variations in the occurrence in different localities and different years.

More arguments for the conception that in Umbelliferous plants both a low germination percentage due to embryoless seeds and a reduction in the seed production due to a check in fruit development may result from the feeding of bugs belonging to the genus *Lygus* are to be found in the literature.

FLEMION and HENRICKSON (3) detected embryolessness in seed grown in Holland, Denmark, North Africa, Egypt, Norway and several areas in the United States. FLEMION, POOLE and OLSON (4) found that in addition to embryolessness the feeding of *Lygus oblineatus* on flowering umbels of dill resulted in a reduction in the seed yield. In Canada, HANDFORD (7) recorded a reduction in the seed production in the carrot when *Lygus campestris* was found in the fields.

For the sake of completeness we mention here the articles by FLEMION and OLSON (5), FLEMION and MAC NEAR (6), WAGN (11) and ROBINSON (8). These articles came into our possession when we had almost finished our work, and will be treated in the discussion of our results.

EXPERIMENTAL RESULTS

Our experiments have been based on the following working hypothesis:

The feeding of *Lygus campestris* on umbels of the carrot may cause a reduction in the seed yield due to a check in the development of the young fruits and a reduction in the germination percentage due to embryolessness. The former injury is identical with the "seedless umbels" disorder.

To prove this, bugs of the species *Lygus campestris* were brought upon the umbels of carrot plants. Preliminary experiments were started in 1953. The next year our first definitive experiment was carried out and it was repeated in 1955. We proceeded as follows. Carrot plants of the variety Amsterdam Forcing were brought into a greenhouse with a constant temperature of 17°C. When the main flower stem had been formed, the plants were transferred to small insect-proof glasshouses; at least four plants were placed in each glasshouse. Flies of the species *Phormia terranova* R.D. were introduced as soon as the first umbels started flowering to ensure satisfactory pollination. The flies were supplied to the plants till the end of anthesis of all the main umbels. Only these primary umbels have been examined. The plants were divided into three groups:

- A. Plants with bugs added during anthesis, i.e. during periods from ± 1 week before until ± 12 days after the first flowers of the main umbel had opened.
- B. Those with bugs added after the seeds had set, i.e. ± 3 weeks after the beginning of anthesis of the main umbel.
- C. The control plants, without bugs.

The bugs were kept on the plants until harvest. At about the end of August when the seeds of the main umbels were ripe, they were harvested and examined immediately. The experimental results in 1954 and 1955 agree well, and can consequently be discussed together.

It was established that by adding bugs of the species *Lygus campestris* to umbels of the carrot, symptoms could be produced that were identical with those of the seedless umbels observed in the field. Especially in group A, where bugs had been added before or during anthesis, most of the umbels had the appearance characteristic of this disorder. Many fruits were undeveloped or only partially so, the peduncles of the umbellets standing upright, the outer ones being turned inward over the central ones. The affected umbels differed appreciably in appearance from the normally developed umbels of group C, where no bugs had been added. Here the umbels had flattened out because of the large number of well-developed fruits the long setae of which gave a woolly and light coloured appearance to the upper surfaces of the umbels. In group B, where the bugs had been added after fertilization, the development of the umbels seemed to be as good as in the control plants.

After a more detailed examination of the seed umbels the following facts were established. Under-sized fruits also occurred in the control group C. Here they were not conspicuous, because sufficient normal seeds were present to give the umbel the normal full appearance. The under-sized fruits in this group were all very small, being no larger than the ovaries in just expanded flowers. Also in field-grown wild and cultivated carrot plants these undeveloped ovaries have been found in seed umbels (1).

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Apparently in carrots a number of ovaries always remain undeveloped due to insufficient pollination, defective fertilization or possibly some other cause. In group A only few normal seeds were produced and the majority of the fruits remained small. Among them were the undeveloped ovaries just described, but also an appreciable number of partially developed fruits. In group B the number of normal seeds was quite large, the under-sized fruits mainly consisted of undeveloped ovaries and to some extent of partially developed fruits.

The effect of *Lygus* bugs on seed development in the carrot is depicted in Fig. 1. The upper two photographs show two typical seed umbels from treatment A (bugs added before and during anthesis). Lower left: an umbel from treatment B (bugs added after anthesis) and lower right: an umbel from treatment C (control).

Attempts were made to assess quantitatively the detrimental effect of *Lygus* on the seed production of the carrot. After the primary seed umbels of the 1955 experiment

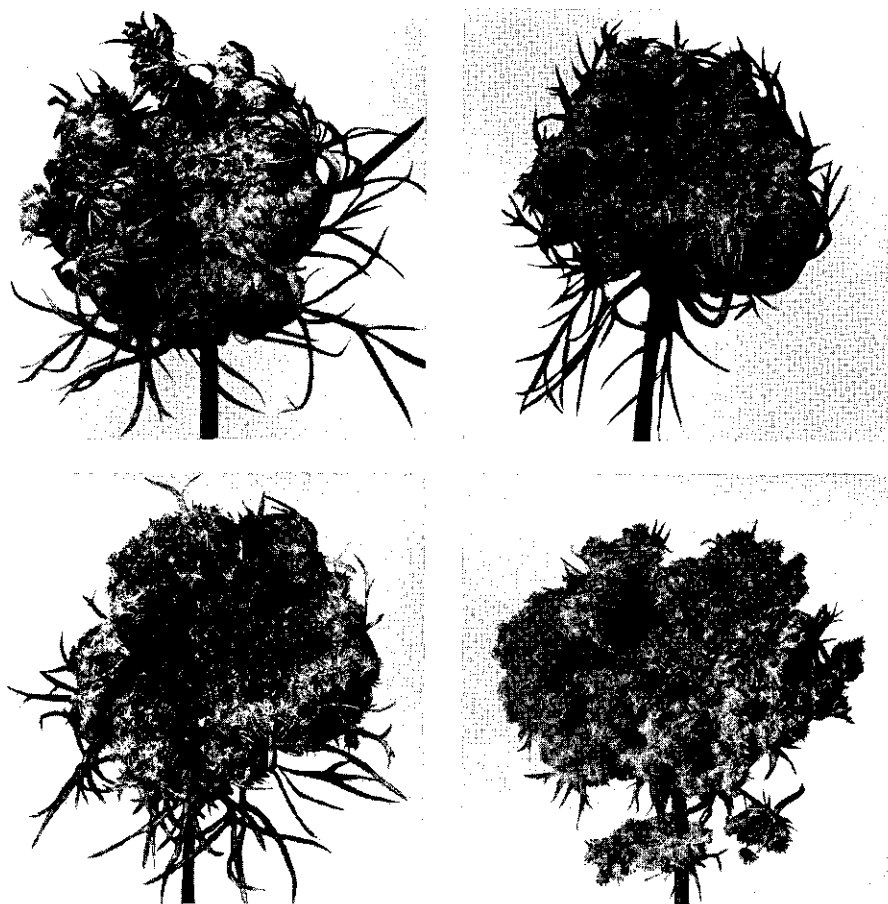


FIG. 1. PRIMARY UMBELS FROM THE 1954 EXPERIMENT. ABOVE: TWO UMBELS FROM TREATMENT A (BUGS ADDED BEFORE AND DURING ANTHESIS). LOWER LEFT: UMBEL FROM TREATMENT B (BUGS ADDED AFTER ANTHESIS). LOWER RIGHT: CONTROL

had been harvested, the normally developed seeds were removed and counted and then the under-sized fruitlets remaining on the umbels were also counted. Each ovary can develop into a bilocular schizocarp, consequently it has the potential ability to produce two "seeds". Addition of the number of under-sized fruits and half the number of normal seeds gives the total number of ovaries per umbel. The results of these counts are given in Table 1.

TABLE 1. THE 1955 EXPERIMENT. EFFECT OF *Lygus* BUGS ON SEED PRODUCTION OF PRIMARY UMBELS IN THE CARROT VARIETY *Amsterdam Forcing*

Treatment	Serial no. of plant	Observed per primary Umbel		Averages per treatment		
		Number of ovaries	Number of normal seeds	Number of ovaries	Number of normal seeds	Number of seeds per ovary
a	b	c	d	e	f	g
A <i>Lygus</i> added a few days before or during anthesis	1	1572	513	1545	432	0.28
	2	1440	469			
	3	1534	482			
	4	1393	687			
	5	1719	322			
	6	1628	321			
	7	1529	227			
B <i>Lygus</i> added after anthesis	8	2037	436	2084	767	0.32
	9	1903	1092			
	10	2181	693			
	11	2185	759			
	12	2101	591			
C Control. No <i>Lygus</i> added	13	2098	1034	2054	910	0.44
	14	2132	1129			
	15	1994	914			
	16	2036	688			

Column f of the table shows that the average number of normal seeds per umbel, which is 910 in the control, is reduced to 413 when the bugs are present before and during anthesis and to 767 when they are brought on the plants after the setting of the fruits.

The larger reduction in the seed production in treatment A, as compared with treatment B, must be ascribed to the fact that the flowers are more easily injured by the feeding of the bugs than are the young fruits. From column e in Table 1 it may be seen that the average number of ovaries per umbel is reduced by about 1/4 in treatment A, but that in treatment B it is the same as in the control. Apparently a number of the flowers have been injured to such an extent that they were killed and abscised in an early stage. However, the high sensitivity of the flowers to attacks by *Lygus* bugs is not only shown by the abscission of flowers but even more by the arrested growth of the young fruits. In column g of Table 1 it is shown that the number of normal seeds produced by each ovary amounting to 0.44 in the control, is reduced to 0.32 in treatment B and even to 0.28 in treatment A.

As has already been mentioned in the general description of our results a number

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of the ovaries in the umbels of carrot show no development at all. From the data in Table 1 we see that their number is unexpectedly high. The number of seeds per ovary in the control group (C) is 0.44 where it could have been as high as 2.0.

To find out whether *Lygus* bugs on umbels of carrots are responsible both for a reduction in yield and in germination capacity, we have subjected seeds obtained from the 3 treatments of both experiments to a germination test. Fifty apparently normal seeds from each primary umbel were placed on moist filter paper in Petri dishes. The Petri dishes were incubated in the dark for 14 days at a temperature of 25°C. After determining the germination percentage, the seeds that had not germinated were cut into halves under a dissecting microscope and their contents examined.

TABLE 2. THE 1955 EXPERIMENT. REDUCTION IN GERMINATION PERCENTAGE IN CARROT SEEDS CAUSED BY *Lygus campestris*

Treatment	Serial no. of plant	No. of seeds tested	Number of seeds per plant			Average percentage per treatment		
			Germinated	Not germinated		Germinated	Not germinated	
				Healthy	Injured		Healthy	Injured
a	b	c	d	e	f	g	h	i
A <i>Lygus</i> added before and during anthesis	1	50	31	3	16			
	2	50	26	4	20			
	3	50	27	4	19			
	4	50	6	9	35	38	11	51
	5	50	9	4	37	(33) ¹⁾	(12)	(55)
	6	50	22	3	25			
	7	50	11	11	28			
B <i>Lygus</i> added after fruit set	8	50	24	6	20			
	9	50	26	5	19			
	10	50	37	4	9	65	8	27
	11	50	33	6	11	(69)	(5)	(26)
	12	50	39	1	10			
	13	50	36	1	13			
C Control. No <i>Lygus</i> added	14	50	48	2	-			
	15	50	47	2	1	94	4	2
	16	50	46	2	2	(88)	(6)	(6)

¹⁾ The figures in parentheses relate to the experiments of 1954.

In Table 2 the results of the germination test are given. Column g shows that in 1955 the mean germination percentage of 94 in the control was reduced to 38 when the bugs were present on umbels during the flowering stage and to 65 when the bugs were added after anthesis. This is in close agreement with the results of 1954 when the corresponding figures were 88 %, 33 % and 69 %. In this respect, too, *Lygus* proves to be more harmful in the earlier stages of development of the umbels than later on.

In the non-germinating seeds 3 categories could be distinguished: 1. Seeds containing a normal endosperm and a normal or sometimes somewhat smaller embryo. "Dormant" seeds have been repeatedly found in Umbelliferous plants (8). The slight increase in the percentage of these "healthy" seeds in treatment A of Table 2 is probably due to the fact that seeds with small injuries may have been erroneously placed

by us in this category. 2. Embryoless seeds possessing a normal endosperm, which encloses a cavity instead of the small embryo. 3. Decayed seeds which sometimes show fungal growth on the seed-coat and the contents of which have been converted to a gelatinous or pasty substance. The latter two kinds of seeds are classed together as "injured" seeds in Table 2. Only in the treatments where *Lygus* bugs have been added

TABLE 3. THE 1955 EXPERIMENT. PRODUCTION OF OVARIES, SEEDS AND VIABLE SEEDS BY *Lygus* INFESTED CARROT PLANTS EXPRESSED AS PERCENTAGES OF THE PRODUCTION BY CONTROL PLANTS

Treatment	Ovaries	Seeds	Viable seeds
A. <i>Lygus</i> added before and during anthesis	75%	47%	19%
B. <i>Lygus</i> added after anthesis	101%	84%	58%

do these seeds occur in appreciable numbers, and consequently must be regarded as the typical results of the feeding of these insects.

In order to assess the total damage that can be caused by *Lygus campestris* to the seed production of carrots we have summarized the data given in Tables 1 and 2. This summary is presented in Table 3, in which for the treatments A and B of the 1955 experiment the average number of ovaries, full-grown seeds and viable seeds is expressed as a percentage of the numbers produced in the control.

Table 3 shows clearly that, depending on the moment at which the attack is made, the *Lygus* bug causes a reduction in the total yield of carrot seed varying from 16% to 53%, while the yield of viable seeds is reduced by 42% to 81%.

In addition to the above experiments, a microscopical investigation was made of the internal condition of under-sized fruits from affected umbels both of field-grown plants and of plants grown in our *Lygus* experiments. The material was fixed in Navashin modified by RANDOLPH, or in F.A.A. Then it was embedded in paraffin and cut with a microtome. The sections were stained with haematoxylin using the method of HEIDENHAIN or with safranin and Fast Green, and then mounted in Canada balsam.

On examining the slides we found that in most of the undeveloped ovaries fertilization had not taken place; any pollen grains that were present on the stigma had not germinated or the pollen tubes had not penetrated far into the stigma. In slightly larger but backward fruits, however, fertilization had taken place. The embryo sac contained a many-celled endosperm and often a very small filamentous embryo. The pericarp and integuments did not show any abnormalities, but the endosperm presented a very atypical appearance due to local disorganisation of the endospermal tissue. In some preparations only a small part of the endosperm was affected, but in others the disintegrated spots coalesced so that only remnants of the endosperm and embryo were visible. When staining with haematoxylin the affected parts were conspicuous for a marked absorption of the stain. With safranin and Fast Green, however, they were not more markedly stained than the normal endosperm, and looked like cavities filled with remnants of tissue (Fig. 2).

A fact which was important to us was that the same picture was shown both by field material and material from our *Lygus* experiments, and that this picture was not

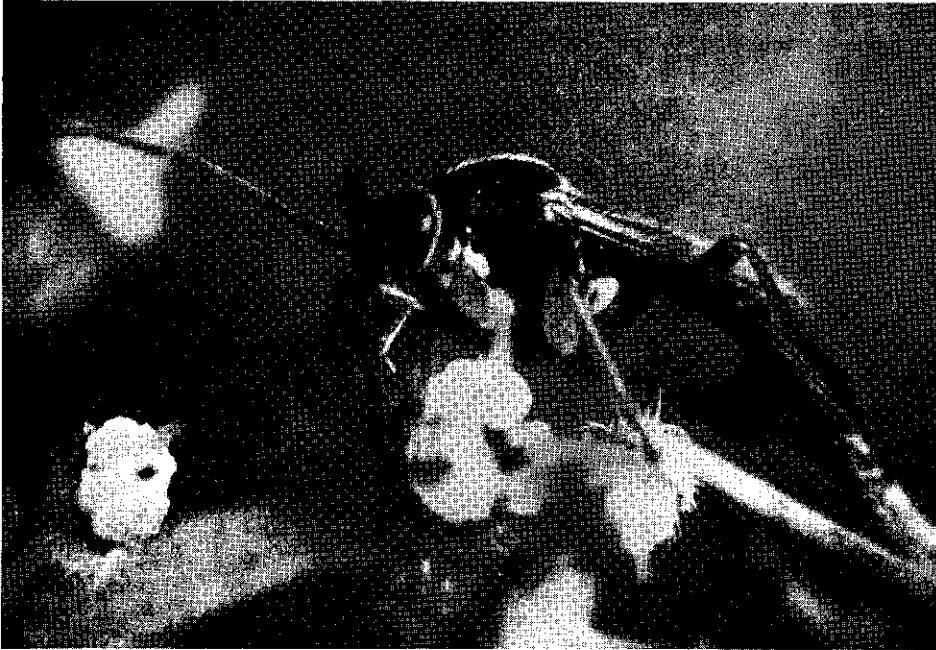


FIG. 4. *Lygus campestris* FEEDING ON YOUNG FRUIT OF THE CARROT, $\times 15$.

inconsistent with the supposition that the destruction of the endosperm was caused by the feeding of bugs.

At first we did not find any perforations of the pericarp caused by the feeding of bugs. When observing the behaviour of bugs on umbels we noticed, however, that *Lygus campestris* feeds not only on the peduncles of umbels and on pedicels, but also on ovaries and fruits. We often saw that bugs examined the surface of a fruit for a little while and then, by bending the proboscis, uncovered the stylets and inserted them into the tissue. Fig. 4 shows a bug feeding on a young fruit. The stylets and backwards hinging doubled proboscis are clearly visible. We could not confirm the statement by WAGN (11) that the bugs prefer a particular region of the fruit for feeding.

A close examination of slides stained with safranin revealed that

- a. disintegration of the contents of the ovule was often accompanied by perforations of the pericarp;
- b. both seeds of a fruit showed feeding punctures;
- c. feeding punctures were present both on material from our bug experiments and on field material (Fig. 3).

DISCUSSION

From the results of these experiments we may conclude that our working hypothesis is correct. This means that in the Netherlands *Lygus campestris* must be regarded as the cause both of the reduction in seed yield of carrots known as the "seedless umbels" disorder, and of the low germination percentage of carrot seed well-known of old.

In addition our experiments have shown that the two forms of bug injury become more serious when the carrot umbels are affected at an earlier stage of development.

The theory mentioned earlier in this paper to the effect that both types of bug injury may be caused by several species of the genus *Lygus* in different Umbelliferous crops is supported both by the results of our own experiments and by data in the following publications, which did not come into our possession until we had nearly finished our investigations: WAGN (11) found that the seed of caged carrot umbels showed a reduction in germination percentage due to embryolessness when the cages contained bugs of the species *Lygus campestris* L., *L. kalmi* L., *L. pubescens* REUT. or *L. pratensis* L. In caged plants of Umbelliferous crops, including carrot, FLEMION and OLSON (5) noticed a reduction both in germination percentage and in seed yield when bugs of the species *Lygus oblineatus* SAY were present. Finally FLEMION and MAC NEAR (6) found that in dill (*Anethum graveolens* L.) and fennel (*Foeniculum dulce* MILL.) *Lygus oblineatus* caused a reduction both in germination percentage and in seed production, and that both forms of injury became more serious when bugs were added at an earlier developmental stage of the umbels.

In view of the probably general occurrence of the discussed injuries it is of interest to attempt a rough estimate of the damage caused to seed production in practice.

Exact data on the reduction in seed yield from field-grown plants are not available. However, on combining the figures obtained in our experiments with the field data given by HANDFORD (7) it will be seen that in carrots *Lygus campestris* causes a loss of about 40 % of the total yield. To be on the safe side we will assume for our calculation a reduction of only 25 %. On the germination percentage of field-grown Umbelliferous crops sufficient figures are at hand.

TABLE 4. GERMINATION STANDARDS FOR SOME UMBELLIFEROUS CROPS

Crop	U.S.A.	Netherlands
Carrot (<i>Daucus carota</i> L.)	55	61
Celery (<i>Apium graveolens</i> L.)	55	75
Parsley (<i>Petroselinum sativum</i> HOFFM.)	60	76
Parsnip (<i>Pastinaca sativa</i> L.)	60	84

Table 4 shows the standard germination percentages of 4 Umbelliferous crops required in the United States and in the Netherlands. The figures are derived from the articles of ROBINSON (8) and from the Dutch Horticultural Guide (10) respectively.

In our calculations we will restrict ourselves to the damage caused to seed production of carrots in the Netherlands. We assume that the reduction in yield and germination averages 25 % and 40 % respectively. For the production of carrot seed in the years 1951-1954 the following average figures are supplied by official sources (9, 10): Area 133 hectares, yield 60,000 kg (viable seed only 36,000 kg), value 213,000 guilders. In the absence of *Lygus* bugs only 75% × 60%, i.e. 45 % of the present acreage would be needed for the production of the same amount (36,000 kg) of viable seed. In this manner costs would be reduced to about one half.

As stated above the outcome of this calculation should be regarded as a rough estimate. Nevertheless this estimate is reliable enough to conclude that the damage is considerable. Since Umbelliferous crops are grown in most parts of the world, and

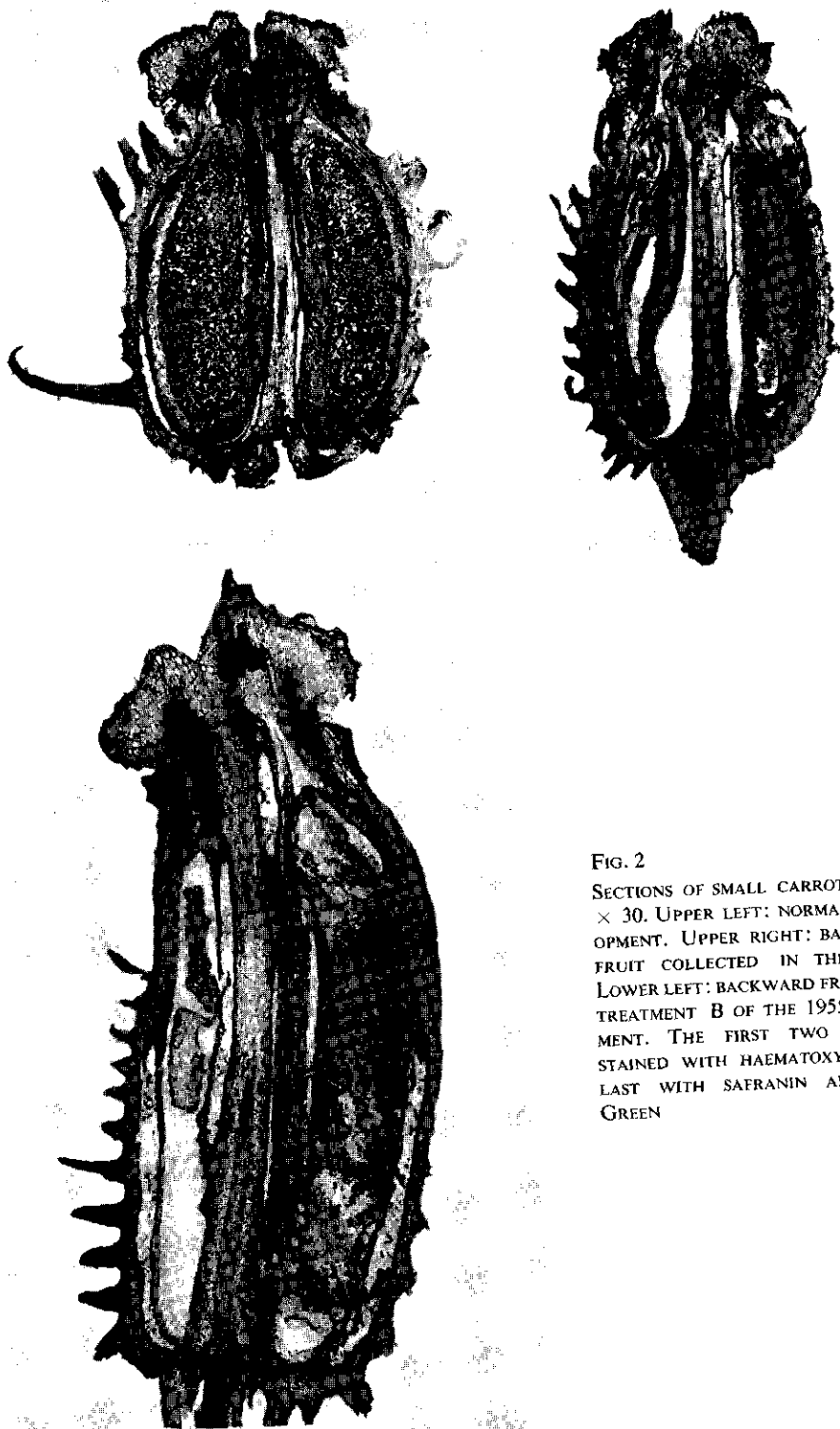


FIG. 2

SECTIONS OF SMALL CARROT FRUITS,
× 30. UPPER LEFT: NORMAL DEVELOPMENT.
UPPER RIGHT: BACKWARD FRUIT COLLECTED
IN THE FIELD. LOWER LEFT: BACKWARD
FRUIT FROM TREATMENT B OF THE 1955
EXPERIMENT. THE FIRST TWO SECTIONS
STAINED WITH HAEMATOXYLIN, THE
LAST WITH SAFRANIN AND FAST
GREEN

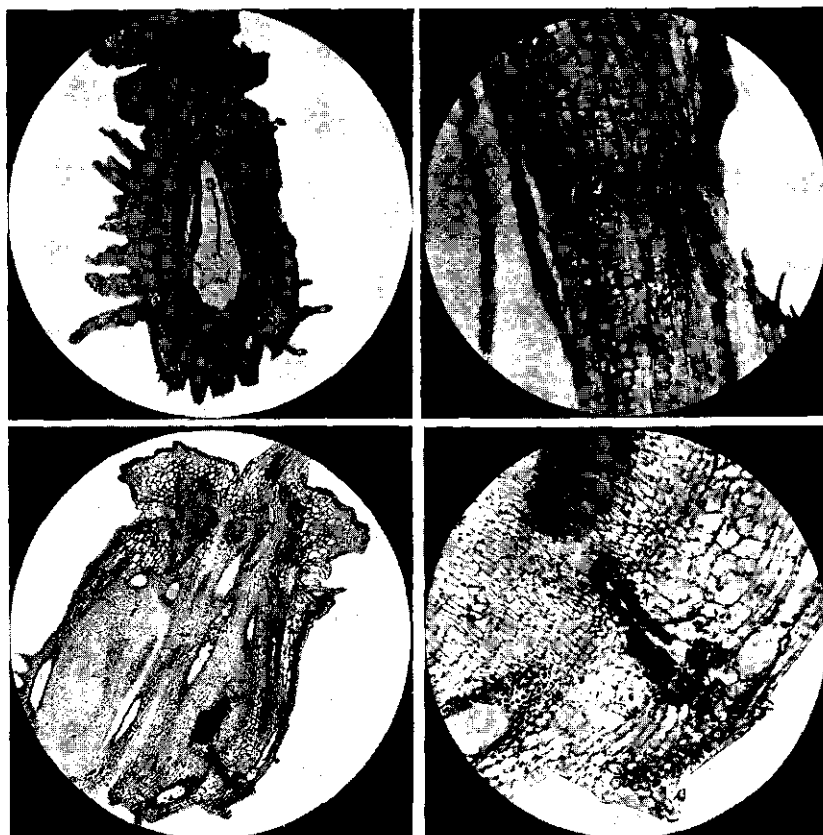


FIG. 3. SECTIONS OF SMALL CARROT FRUITS SHOWING FEEDING PUNCTURES BY *Lygus*.
UPPER LEFT: BACKWARD FRUIT COLLECTED IN THE FIELD, $\times 20$. UPPER RIGHT:
THE SAME, $\times 80$. LOWER LEFT: BACKWARD FRUIT FROM TREATMENT A OF THE 1955
EXPERIMENT, $\times 20$. LOWER RIGHT: THE SAME, $\times 80$.

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bugs of the genus *Lygus* have a wide distribution, we may assume that the damage caused by bugs in all parts of the world represents a very high amount. To conclude with we should therefore like to draw attention to the desirability of achieving in the near future an effective control of *Lygus* bugs on the flower heads of Umbelliferous crops by the co-operation of entomologists and phytopathologists.

SUMMARY

Research was carried out into the cause of a disorder occurring in seed plants of carrots characterized by backward fruits and resulting in a reduced seed yield.

It appeared that both fertilization and initial development of the endosperm and embryo were normal, but that later on a sudden check in the growth of the fruit occurred, accompanied by disintegration of the contents of the ovule.

Bugs of the species *Lygus campestris* L. were found to occur in considerable numbers on the seed plants of carrots. In experiments a reduced seed production with the same macroscopic and microscopic symptoms could be obtained when bugs were added to caged carrot umbels.

It could be established that the presence of bugs caused not only a reduction in the seed production due to abortion of endosperm and embryo, but also a reduction in the germination percentage of the full-grown seeds due to embryolessness.

When the bugs were brought on the umbels before or during anthesis the reduction in seed viability and especially the reduction in seed production proved to be more serious than when the bugs were added after anthesis.

SAMENVATTING

*Verminderde zaadopbrengst van de peen en kiemkrachtsvermindering van het zaad als gevolg van het voorkomen van de wants *Lygus campestris* L.*

Een onderzoek is ingesteld naar de oorzaak van een kwaal van zaadplanten van peen, welke gekarakteriseerd is door het klein blijven van de vruchten en welke aanleiding geeft tot een verminderde zaadopbrengst.

Het onderzoek bracht aan het licht, dat bevruchting en de aanvankelijke ontwikkeling van endosperm en embryo normaal verliepen en dat daarna plotseling stilstand in de groei van de vrucht plaats vond, gepaard gaande met gedeeltelijke of totale desintegratie van de inhoud van de zaadknop.

Wantsen van de soort *Lygus campestris* L. bleken op zaadplanten van wortels veelvuldig voor te komen. In proeven, waarbij deze wantsen opzettelijk op geïsoleerde bloemschermen van peen gebracht werden, trad eveneens een verminderde zaadproductie op met de hierboven vermelde macroscopische en microscopische symptomen.

Vastgesteld kon worden dat bij aanwezigheid van wantsen niet alleen een vermindering van de zaadopbrengst berustend op abortie van endosperm en embryo optrad, maar ook een verminderd kiempercentage bij de normaal uitgegroeide zaden berustend op embryoeloosheid.

Wanneer de wantsen voor of tijdens de bloei op de schermen gebracht werden, waren de kiemkrachtvermindering en vooral de vermindering in de productie van volgroeide zaden ernstiger dan wanneer de dieren na de bloei werden toegevoegd.

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- 29-11-'50. Uitslag Practijkproeven Platronde en Ronde Kroten 1949-1950.
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- 4-11-'52. Uitslag Practijkproeven Vroege Rijspeulen 1951-1952.
- 25-11-'52. Uitslag Practijkproeven Lange Kroten 1951-1952.
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- 13-5-'53. Uitslag Practijkproeven Bewaar Rode Kool 1951-1952.
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- 18-12-'53. Uitslag Practijkproeven Herfst Witte Kool 1952-1953.
- 3-6-'54. Uitslag Practijkproeven Bewaar Witte Kool 1952-1953.
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¹⁾ Zolang de voorraad strekt kunnen deze publikaties franco worden toegezonden, na ontvangst van het vermelde bedrag op giro no. 425340 van het Instituut voor de Veredeling van Tuinbouwgewassen, S. L. Mansholtlaan 15 te Wageningen onder vermelding van wat verlangd wordt; ook bestaat de mogelijkheid deze publikaties uit de bibliotheek van het I.V.T. te lenen.
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