linoleic acid synthesis pathway described here is under investigation. The findings together with an assessment as to how this system might best be used in commercial open-pollinated and hybrid cultivars will be presented in a subsequent paper.

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T1982GEN23

SELECTION IN SUNFLOWERS FOR RESISTANCE TO RUTHERGLEN BUG *(NYSIUS VINITOR).*

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ABSTRACT

Because of the damage done to sunflower crops in Australia by the Rutherglen Bug, resistant varieties are required. Plants in populations of wild roadside sunflowers which have been exposed to the bug for many years tolerate and resist attack. In progenies of crosses between wild selections and cultivated sunflowers, variation for resistance was observed and selections made. From these, elite families were selected on germination percentage and oil content. The resistance genes are being transferred into a suitable background to allow their use in commercial varieties.

INTRODUCTION

The Rutherglen Bug (*Nysius vinitor* Berg.) is a member of a genus of plant-attacking *Hemiptera* with world-wide distribution (Slater, 1964). *N. ericae*, the false chinch bug is a serious pest in semi-arid parts of the United States (Milliken, 1918), while the Wheat Bug (*N. huttoni*) damages the New Zealand wheat crop (Burnett, 1976).

In Australia, the Rutherglen Bug breeds in temporary habitats, particularly on seeds of annual plants (Kehat and Wyndham, 1972) and flies to new areas in response to adverse conditions (Kehat and Wyndham, 1973). Thus as winter-growing plant species mature large populations of bugs migrate to sunflower crops.

It is often a week or two before flowering is expected when the bug is first noticed, clustered on the peduncles of the developing buds. Soon wilting can be observed and the reproductive apex may die. Control is difficult because insecticides used about flowering time can kill pollinating insects and the highly mobile Rutherglen Bug can rapidly recolonize sprayed areas. Thus an attack just before flowering causes substantial losses to the farmer.

After the plant has flowered the bugs live on the capitulum, among the bracts and on the developing seeds. It is thought that seed attack induces an increase in free fatty acids which detract from oil quality. The effects of Rutherglen Bug on oil content and seed viability do not appear to be documented. But despite the lack of knowledge of the bug and its effects, it does constitute a serious pest against which it would be useful to have resistant varieties. Indications that this might be possible were provided by Greaves and Rochford (1946) who observed variation in susceptibility among potato varieties. More particularly, encouragement is provided by the success in breeding for resistance to the related chinch bug, *Blissus leucopterus* Say. in maize (Flint, 1921), in sorghum (Snelling *et al.*, 1937) and in wheat (Jones, 1937). In this paper we will describe efforts to locate and develop sources of resistance to Rutherglen Bug in sunflowers.

METHODS

Although the genus *Helianthus* is not native to Australia, naturalized populations of *H. annuus* are widely distributed. They exhibit the dominant wild-type branching from the lower nodes of the wild species rather than the solitary heads and recessive branching which might be expected in escapes from cultivation. Nevertheless in some areas considerable introgression between wild and cultivated forms can be

observed.

During a severe plague of Rutherglen Bug early in 1979 individual roadside plants were observed to either carry low populations of the pest or to lack evidence of damage despite the insect's presence. Mature seed was taken from aparently resistant plants. In addition, samples of pollen were collected from selected wild plants to develop a mixed pool of pollen. This was applied to receptive stigmas of cultivated sunflowers in a nursery to provide F_1 seed. This seed together with that from 14 wild populations was sown in a glasshouse in 1979 to allow further hybridization between wild and cultivated forms. This gave rise to F_1, F_2, F_3, BC1 and BC2 families.

In October 1980, some 120 of these families together with 10 standard varieties and inbreds were sown in 2-row plots, 5 meters long (each plot containing about 50 plants) in two replications in the field at Canberra. In December 1980 there was a massive invasion of Rutherglen Bugs particularly from maturing *Brassica* weeds in the vicinity. The attack became severe as the flower buds were expanding and susceptible plants and rows were devastated. All the buds of inbred AHA89, for example, were killed.

Tags were placed on those plants which either attracted very few bugs, or which, despite some infestation, apparently did not suffer damage. Soon after flowering, plants which carried very few bugs on the capitulum and seeds were noted. During seed development these marked plants were observed on two further occasions to monitor infestation and evidence of damage.

Seed from the selected plants was evaluated by the following means:

1. Oil content. Seed oil in the whole seed in each line was determined by using wide band nuclear magnetic resonance (Newport equipment) with sunflower oil as a standard.

2. Germination percentage. From each of the lines, two replicates each of 25 seeds were germinated in flats of soil in a glasshouse. Counts of seedling emergence were made at approximately 14 days.

3. Seedling damage. Cotyledons of seedlings in flats were rated for evidence of necrotic regions, discoloration and uneven margins which might be related to bug attack of the development seed.

4. Seedling vigour. Seedlings were rated for vigour as a possible indicator of poisoning by products produced as a result of Rutherglen Bug attack.

5. Free fatty acid content of Oil. Free fatty acids were determined on the extracted oil by the AOAC method for crude oils. Results were reported as percent free fatty acids, expressed as oleic acid.

6. Water Soluble protein content of seed. Total nitrogen was determined on the fat-free meal using a micro-Kjeldahl procedure. A micro-biuret method was used to determine water soluble protein using bovine albumin as a standard.

RESULTS AND DISCUSSION

A severe attack by Rutherglen Bugs in the field late in 1980 made it possible to observe the reaction of individual plants in a nursery of segregating (wild x cultivated) sunflower families. From a population of 12,000, 349 selections were made before or about flowering time. Some tolerated high infestations of the bug on the peduncle and petioles of upper leaves without showing evidence of wilting. Others were selected on the bugs' non-preference (Painter, 1951) for them. It is unlikely that this category includes evidence for antibiosis since insects are mobile and need not remain on particular plants. During seed development, appearance of wilting symptoms or increase in bug numbers caused some earlier selections to be rejected. Ultimately 197 selections (all open pollinated) were harvested. These constitute 1.6% of the original population.

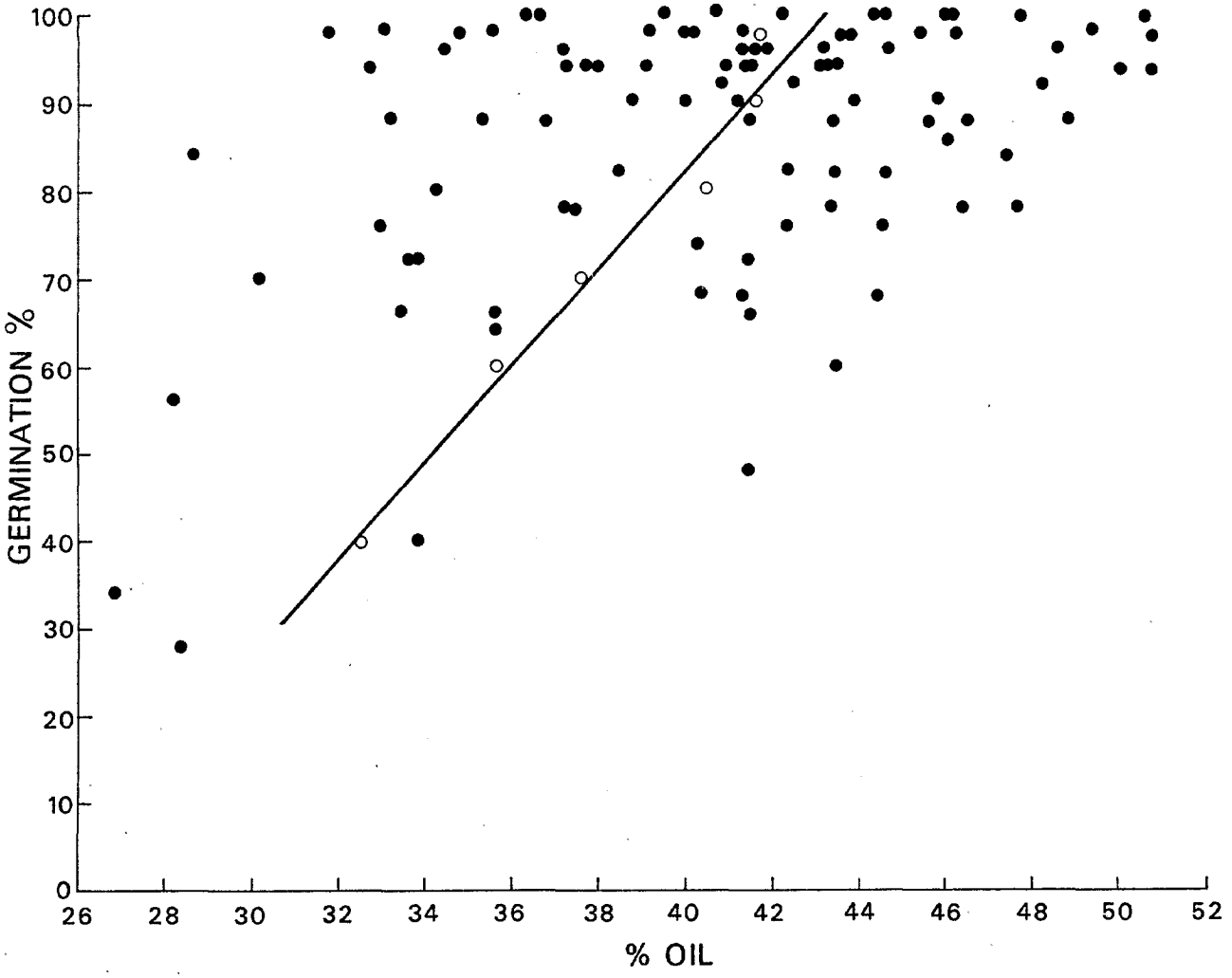
Despite the obvious field resistance shown by the selections, it was important to determine whether or not they had sustained damage. The seed germination percentage was most useful in this regard. About 30% of the selections exhibited a viability of more than 95% and were thus verified as resistant to the Rutherglen Bug. A further 30% plants provided seed which showed 85 — 95% germination suggesting that there had been some damage. The balance however, with less than 85% viability, and as low as 26%, suggests that field selection alone was inadequate to exclude bug-damaged plants.

But is it possible to go further, do symptoms on cotyledons make it possible to more positively exclude damaged plants? While some additional evidence could be collected by observing cotyledons, there was a high correlation (0.95) between percentage germination and percentage of plants with undamaged cotyledons. It follows that the effort involved in growing seedlings to observe evidence of damage is not warranted when a simple germination test serves to identify virtually the same sets of lines.

Assessment of seedlings on vigour was hazardous. Lines which germinated more rapidly or had bigger seeds than others tended to score best in vigour ratings. It was concluded that vigour could provide misleading information and therefore should not be used.

Another easily measured parameter is oil percentage in the seed. But in the 197 lines selected the oil percentage varied between 25 and 52%. The oil percentage in a sample of the selections is shown in relation to germination percentage in figure 1. A wide scatter is evident but to help partition this between genetic and Rutherglen Bug effects the germination data were grouped. The mean oil percentage was determined for selections germinating 25-55, 55-65, 65-75, 75-85, 85-95 and greater than 95%. The germination percentage was thus shown to be related to oil percentage as $Y = 5.55\ X - 139.8$ and with a correlation coefficient of 0.97.

Figure 1. Relationship between germination percentage and oil content in seed of selected plants.



Thus despite the wide segregation for oil percentage due to plant characteristics, most likely hull/kernel relationships, the presence of the Rutherglen Bug as reflected in germination percentage, appears to have had an adverse effect on oil content.

It seems that plants with seed germinating above about 95% are resistant to the bug. Whether this alone is used or whether only those plants with high oil content as well should be selected must depend on the breeding methods used and the breadth of the genetic base required. The range in the background of 49 selections is shown in table 1. It may be significant that resistance genes were detected in a number of wild populations. On the one hand it may suggest that many of these populations arose from a common source, or alternatively, that the insect pressure has been such that is has provided a stringent filter favoring those plants with superior fitness in the presence of bugs.

Table 1. Origin of wild parents of 49 selections*

Pollen (bulked)	16
Coonabarabran	9
Mulwalla	6
Premer	5
Coolah	3
Narrabri	3
Gilgandra	2
Mullaley	2
Peak Hill	2
Gunnedah	1

*based on field resistance and seed of more than 40% oil and 95% germination.

There is another aspect to figure 1 which indicates that Rutherglen Bug attack reduces oil percentage. These selections exhibited little or no field damage and most did not carry large bug populations, probably too few on an individual plant basis, to have warranted use of insecticides in a commercial field. This suggests that even low levels of bug attack can reduce substantially the oil percentage of susceptible sunflower crops. Perhaps the full extent of Rutherglen Bug damage is yet to be described.

To seek a relationship between germination percentage, which appears to reflect Rutherglen Bug damage, and other verifying criteria, the presence of free fatty acids in the oil was considered. Five plants were selected on their apparent field resistance and high levels of seed germination, and six "susceptible" plants were chosen on their low seed germination percentage (they also had low oil percentage) despite evidence of field tolerance. And three glasshouse-grown plants which had not been exposed to the bug were used as controls. The findings are shown in Table 2. Among the plants selected as susceptible to the bug were some that had high levels of free fatty acids (group B) while others (group A) did not. However group A plants contained high levels of soluble protein suggesting that protein quality had been altered.

Table 2. Free fatty acid and soluble protein in seed of sunflower plants

	%FFA	% Soluble Protein
Controls	0.77 ± 0.18	1.70 ± 0.62
Resistant Plants	0.73 ± 0.35	1.20 ± 0.96
Susceptible plants		
Overall	3.52 ± 3.46	2.60 ± 1.60
Group A	0.97 ± 0.15	4.03 ± 0.46
Group B	6.07 ± 3.22	1.17 ± 0.22

That the free fatty acid and soluble protein content increased with evidence of bug attack suggests that the seed is affected by an enzyme injected by the insects. This may

indicate a similarity between the nature of the Rutherglen Bug attack and that of the wheat Bug in New Zealand wheat, in which the insect injects an enzyme which denatures the protein in the grain (Burnett, 1976).

Rutherglen Bug damage has been attributed to the bugs sucking the sap under high evaporative demand conditions and thus causing the leaves to wilt (Evans, 1943). Davidson and Peairs (1966) used a similar explanation with respect to *Nysius ericae*. But this does not appear to be the case in sunflower. The resistance to flow through sunflower petioles being attacked by the bug was 5550 bars sec^{-1} cm^{-3} compared with 250 bars sec^{-1} cm^{-3} in unattacked plants (Turner and Downes unpublished). This 22-fold increase in resistance suggests that the bugs are not merely removing sap, they are impeding sap flow, perhaps by the injection of enzymes.

The evidence above suggests that resistance to Rutherglen Bug is available in wild sunflowers. One form of resistance of value is the non-preference type in which the insects are repelled by resistant plants. If on the other hand the bugs attack but the plants are not damaged it is possible that some plant compound, perhaps a tannin, precipitates the protein in the enzyme injected before it damages the plant.

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CHEMICALLY INDUCED MUTATIONS IN SUNFLOWER.

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ABSTRACT

Sunflower seeds of a well adapted, high general combining ability inbred line were treated at varying concentrations and times with the chemicals ethyl methanesulphonate (EMS) and N-methyl-N'-nitro-N-nitrosoguanidine (NTG). Germination tests were conducted on germination paper and in soil in the greenhouse to determine the optimum concentration and time of treatment. Field tests were used to confirm the results of the germination tests. The M_2 and M_3 generations gave individual plants which exhibited earlier bloom date and added resistance to sunflower rust (*Puccinia helianthi*, race 1).

INTRODUCTION

Chemically induced mutations, a supplement ot normal plant breeding methods, were rare in sunflower *(Helianthus annuus)* (Fick, 1978, Voskoboinick and Soldatov, 1974) but in other crop species considerable success was noted. Chemical mutagens affect genic changes as opposed to chromosomal changes from X-ray or gamma radiation (Simons, 1979). Breeding material of seed or flowering plants that was well adapted but lacking one or two desirable characteristics would be excellent source material since it offers the greatest opportunity to be useful in hybrid seed production (Simons, 1979). Several choices of chemical mutagens have been employed and the means of application has varied among researchers (Anonymous, 1977; Voskoboinick and Soldatov, 1974).

The objectives of this study were to develop methodology and test the feasibility of producing functional sunflower mutants by employing chemical mutagens.

MATERIALS AND METHODS

Ethyl methanesulphonate (EMS) and N-methyl-N'-nitro-N-nitrosoguanidine (NTG) solutions were prepared to give concentrations of 50, 250, 500, 2500, and 5000 μg/ml of each chemical. The pH of each mutagen at various concentrations was determined, Table 1. Achenes of the well adapted, high general combining ability inbred, Dahlgren H6B, were treated by vacuum infiltration for one hour under alternating tension of 0 to 1.36 atm. Each concentration of both chemicals and a distilled water check were included in two runs. Germination data were collected from treated seed on split trials by immediately placing on (1) germination paper (rolled towels) after seven days and (2) in greenhouse potting soil after 14 days. A portion of the achenes was force air-dried at 20C for 48 hours and subsamples were tested for germination by the aforementioned methods. Mutagen treated achenes were air-dried for field (sandy soil in Florida) studies.