Naturally Occurring Insecticides in Cruciferous Crops

E. P. LICHTENSTEIN,
D. G. MORGAN, and
C. H. MUELLER

Departments of Entomology and Biochemistry, University of Wisconsin, Madison, Wis.

The stability and occurrence of 2-phenylethylisothiocyanate, an insecticide occurring naturally in the edible parts of turnips, has been investigated. Sixty and 47%, respectively, of the totally recovered 2-phenylethylisothiocyanate from roots of turnip and rutabaga were located within their peelings. Boiling the edible parts of these crops in water resulted in rapid loss of the insecticide by steam distillation. Two cabbage varieties, cauliflower, brussels sprouts, broccoli, kale, mustard, and kohlrabi contained 2-phenylethylisothiocyanate in various amounts as evidenced by gas liquid chromatography and bioassay (houseflies and vinegar flies). Insecticidal activities were encountered only with root tissues. Concentrations of this insecticide in roots ranged from 568 p.p.m. (brussels sprouts) to 83 p.p.m. (cauliflower). Compounds of insecticidal activity—not attributable to 2-phenylethylisothiocyanate—were found in the edible part of radish and leaves of turnip, rutabaga, and garden cress.

THE OCCURRENCE of the mustard oil, 2 - phenylethylisothiocyanate, was first reported by Gadamer (*2*), in 1899, in fresh parts of *Nasturtium officinale* R. Br. and *Barbarea praecox* R. Br. Since then, 2-phenylethylisothiocyanate was encountered in other plant species (*1*, *3*, *7*). Its insecticidal properties were first reported in 1962 (*4*). Since then, further research has been conducted on the occurrence and stability of 2-phenylethylisothiocyanate in the closely related species of turnip (*Brassica campestris* var. *rapa*) and rutabaga (*Brassica napus* var. *napobrassica*). In addition, the occurrence of insecticidal substances in other cruciferous crops grown on insecticide-free soil was investigated.

Procedures

Distribution of 2 - Phenylethylisothiocyanate in Edible Parts of Turnip and Rutabaga. Turnips (Purple Top Strap Leaf) and rutabagas (Laurentian) were grown in 1961 on an insecticide-free Carrington silt loam. After harvest, the edible portion (roots) of the crop was washed with water and peeled. To determine the occurrence and distribution of 2-phenylethylisothiocyanate, whole turnips or rutabagas (roots), as well as their peelings and pulp, were pureed in a food grinder. Extraction, purification, and biological and gas chromatographic investigations of the extracts were accomplished by methods previously described (*4*). The Barber-Colman gas chromatograph equipped with an argon ionization detector was used.

Effect of Boiling Crop Material in Water on Persistence of 2-Phenylethylisothiocyanate. To test the toxicity of crop material after boiling in water, 40 grams of macerated turnip peels were placed in a beaker containing 80 ml. of tap water and covered with a glass plate. The water was boiled for either 5 or 30 minutes, and then filtered off.

Three grams of the boiled plant material were then placed on filter paper on the bottom of each of four small test jars (bioassay jars, $2^3/_4$ inches in diameter and 3 inches deep), into which 50 vinegar flies (*Drosophila melanogastor* Meig.) were introduced. Mortality counts were made 2, 4, 7, and 24 hours later. The same quantity of raw turnip material was used as a control (four replicates). In addition, flies were added to four jars containing only wet filter paper.

The water which had been filtered through paper was collected in a 500-ml. separatory funnel and extracted three times with 100-ml. portions of pentane. After drying with anhydrous sodium sulfate and pooling the pentane fractions, the solvent was concentrated to 10 ml. in a water bath at 45° C. Aliquots were pipetted into bioassay jars, the pentane was evaporated, and 50 vinegar flies were exposed to the dry residue. Mortality counts were made 2, 3, and 24 hours after the flies had been introduced into the jars.

The volatility of 2-phenylethylisothiocyanate with steam was tested in the following manner: 300 grams of turnip peels and 600 ml. of tap water were placed in a 2000-ml. Erlenmeyer flask arranged for distillation. During 1 hour of steam distillation, 40 ml. of aqueous distillate were collected. During an additional hour of boiling, 50 ml. were collected. Each water fraction was extracted with pentane and bioassayed with vinegar flies—measured against a standard of 2-phenylethylisothiocyanate—as previously described (*4*). The distillation condenser was rinsed with pentane which was then tested by bioassay.

Occurrence of Insecticidal Substances in Various Cruciferous Crops. Leaves of turnip or rutabaga were extracted, followed by purification of the extracts by a procedure previously described (*4*). However, the purification on the 10-gram Florisil (60- to 100-mesh) column (20-mm. diameter) was modified by eluting first with 100 ml. of purified pentane and then with 80 ml. of 6% ether in pentane. Each fraction—pentane and pentane-ether (6%)—was then concentrated to 25 ml. Aliquots of the fractions were used for screening tests with vinegar flies to determine if insecticidal properties were present. Following that, various volumes of extracts (pentane-fraction) representing different amounts of leaf tissue were pipetted into duplicate bioassay jars. The solvent was evaporated in each jar, and 50 vinegar flies were exposed for 24 hours to the dry residue.

For gas chromatographic investigations of turnip and rutabaga leaves as well as of edible parts of radish, the crop parts were extracted in a Lourdes homogenizer. Seventy-five grams of fresh plant material were macerated for 5 minutes with a mixture of 150 ml. of acetone-hexane (1:1 by volume). After the crop material had settled, 60 ml. of the solution were transferred to a 500-ml. graduated cylinder containing 190 ml. of water. After the phases were shaken and separated, 5 ml. of the hexane layer were dried over anhydrous sodium sulfate, and an aliquot was used for injection into a gas chromatograph, which in this series was a Jarrell-Ash instrument with an electron affinity detector.

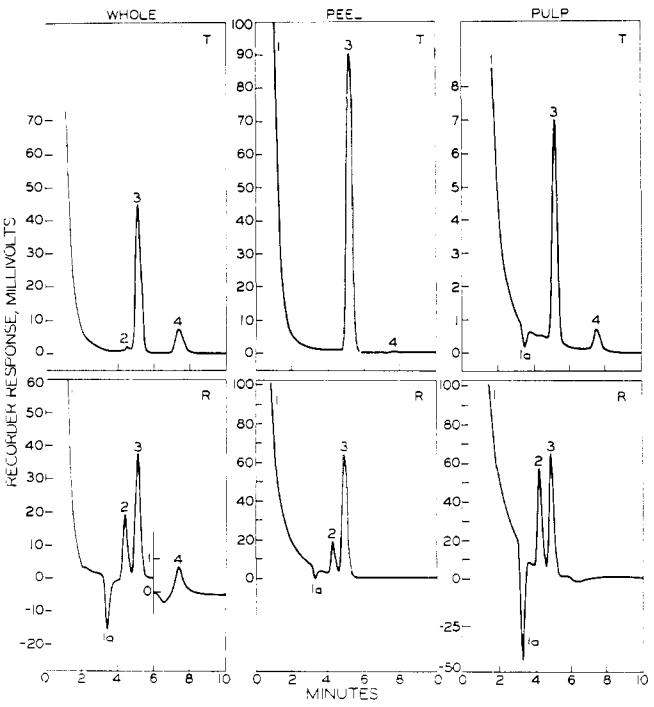

Figure 1. Gas chromatograms of turnip (*T*) and rutabaga (*R*) root extracts showing distribution of various compounds in parts of these crops

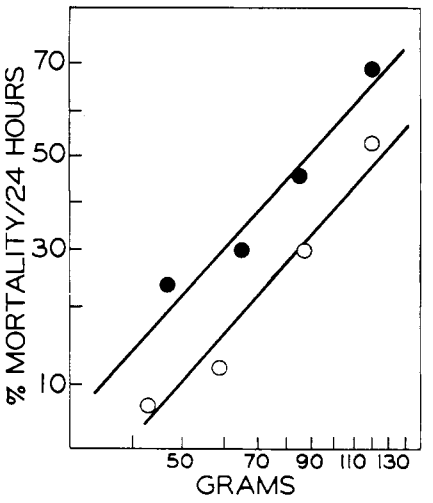

Figure 2. Dosage-mortality curves obtained after 24-hour exposure of *Drosophila melanogastor* Meig. to extracts of turnip (●——●) and rutabaga leaves (o——o)

During the summer of 1962, the following cruciferous crops were grown on insecticide-free Carrington silt loam:

Brassica oleracea var. *capitata*—cabbage (Red Acre)
Brassica oleracea var. *capitata*—cabbage (Wis. Copenhagen)
Brassica oleracea var. *botrytis*—cauliflower (Super Snowball)
Brassica oleracea var. *gemmifera*—brussels sprouts (Long Island)
Brassica oleracea var. *italica*—broccoli (Green Sprouting)
Brassica oleracea var. *acephala*—kale (Dwarf Curled Scotch)
Brassica juncea—mustard (Mustard spinach)
Brassica caulorapa—kohlrabi (Early White Vienna)
Lepidium sativum—garden cress (curled)
Raphanus sativus—radish (Early Scarlet Globe)

To remove soil particles after harvest, the upper edible portions, nonedible leaves, and, where available, roots were washed with water. The parts were then macerated in a food grinder. Crops were tested either by exposing insects directly to the ground material or by extracting the material, followed by gas liquid chromatography.

Insects were also exposed to the dried residue of these extracts. The following extraction procedures were employed using redistilled solvents throughout.

The previously described extraction and cleanup procedure (*4*), using a mixture of acetone-pentane (1:4 by volume) was employed for all top parts ("greens") as well as for roots of kohlrabi and kale. The extracts were concentrated to either 25 ml. or 10 ml., and aliquots were used for both bioassay and gas liquid chromatography

Root material which was hardy and woody (two cabbage varieties, cauliflower, brussels sprouts, and broccoli) was cut with scissors and a knife into pieces 1 to 2 mm. long. Forty grams of the macerated roots were mixed with 80 grams of anhydrous sodium sulfate and dried for 1 hour. The sodium sulfate–root mixture was placed in a 500-ml. Erlenmeyer flask, 200 ml. of acetone were added, and the mixture was stirred for 30 minutes. The supernatant liquid was decanted through glass wool and the recovery volume recorded. The acetone extract was transferred to a 1000-ml. separatory funnel containing 400 ml. of water and 200 ml. of pentane. To reduce formation of an emulsion, 20 grams of sodium chloride were added and the mixture was shaken. After the phases had separated, the water-acetone layer was discarded. The pentane fraction was dried over anhydrous sodium sulfate and either adjusted with pentane to 200 ml. or concentrated to 25 ml. Aliquots of the extracts were used for testing.

Bioassay

When direct exposure methods were used, 3 grams of macerated plant material were placed on wet filter paper in each of eight test jars. Only when roots of the two cabbage varieties, cauliflower, brussels sprouts, broccoli, kohlrabi, and kale were investigated, the macerated material was mixed with water in a 1:1 ratio, yielding a deposit of 1.5 grams of actual plant material on the filter paper. Fifty vinegar flies or 25 houseflies (*Musca domestica* Linn., C.S. M.A. strain) were then introduced into each of four test jars, in which applesauce was provided as food. Insect mortalities were registered over a period of 48 hours.

When extracts were used, aliquots representing 15 grams of root material or 100 grams of tops ("greens") were pipetted into bioassay jars, and the solvent was evaporated at the opening of a fume hood. Fifty vinegar flies were exposed to the dry residue, and mortality counts were made during a period of 48 hours. Applesauce provided the food supply.

After the direct contact bioassay of root extracts had been completed, fine metal screens were placed in front of the dry residue within the same bioassay jars to prevent direct contact of insects with the residue in the following experiment. Vinegar flies were then introduced to test for any fumigant action.

Gas Liquid Chromatography

A Jarrell-Ash gas chromatograph, Model 700, was used, equipped with 100-mc. tritium electron affinity ionization detector (*5*) operated at 28 volts. A 1.22-meter column (3-mm. i.d.) containing Anakrom ABS (acid, alcoholic base, washed and siliconized), 80 to 90 mesh and 1% SE 30-Polyester (neopentyl-glycol, adipate terminated) was conditioned for 48 hours at 200° C. before use. A pressure of 25 p.s.i. of nitrogen gave a flow rate of 100 ml. per minute. The injector temperature was held at 200° C., the column temperature at 152° C., and the detector cell at 208° C. during the run. When extracts of leaves of turnip and rutabaga were analyzed, the column temperature was

Table I. Concentration of 2-Phenylethylisothiocyanate in Different Parts of Turnip and Rutabaga Roots

Root Part	Bioassay[a] P.P.M.	Bioassay[a] R.C.[b]	Gas Liquid Chromatography P.P.M.	Gas Liquid Chromatography R.C.[b]
		TURNIPS		
Whole	40.6 ± 6.1[c]	1.0	37.4 ± 10.0	1.0
Peelings	115.3 ± 22.0	2.8	109.0 ± 25.0	2.9
Pulp	12.0 ± 2.2	0.3	7.0 ± 5.0	0.2
		RUTABAGA		
Whole	37.4 ± 6.2	1.0	30.9 ± 5.0	1.0
Peelings	86.0 ± 5.5	2.3	67.0 ± 25.0	2.2
Pulp	30.3 ± 2.9	0.8	17.1 ± 10.0	0.6

[a] Tested with *Drosophila melanogastor* Meig. against a standard of 2-phenylethylisothiocyanate.
[b] Relative concentration: p.p.m. in whole roots = 1.0.
[c] Standard deviation.

Table II. Effect of Boiling Turnip Peels in Water on the Persistence of 2-Phenylethylisothiocyanate

Exposure Time, Hours	% Mortality of *Drosophila melanogastor* Meig. Exposed to: Wet filter paper only	3 Grams of Turnip Peel Tissue: Raw	Boiled for: 5 min.	Boiled for: 30 min.
2	0	35 ± 11	4 ± 2	0
4	0	90 ± 5	8 ± 5	0
7	0	100	24 ± 2	0
24	42 ± 10		100	0
24[a]	0			0

Exposure Time, Hours	Dry residue of water extract: 5[b]	30[c]
2	50	0
3	90	0
24	100	24

[a] Applesauce added as food supply.
[b] Water in which 40 grams of turnip peels were boiled for 5 minutes.
[c] Water in which 40 grams of turnip peels were boiled for 30 minutes.

Table III. Occurrence of Insecticidal Substances in Cruciferous Crops as Determined by Bioassay and Gas Liquid Chromatography

Crop	Bioassay, % Mortalities after Exposure to: Macerated Tissue[a] D[c]	Macerated Tissue[a] M[d]	Extracts[b], D	Gas Chromatography, P.P.M. Recovered as 2-Phenylethylisothiocyanate
CABBAGE (*Brassica oleracea* var. *capitata*) (RED ACRE)				
Edible part	0/48h[e]	0/48h	0/48h	0.18
Outer leaves	0/48h	0/48h	0/48h	0.34
Roots	50/4h	50/24h	50/15 min[f]	279
CABBAGE (*Brassica oleracea* var. *capitata*) (WIS. COPENHAGEN)				
Edible part	0/48h	0/48h	0/48h	0.06
Outer leaves	0/48h	0/48h	0/48h	0.22
Roots	0/48h	0/48h	50/85 min[f]	139
CAULIFLOWER (*Brassica oleracea* var. *botrytis*) (Super Snowball)				
Edible part	0/48h	0/48h	0/48h	0.13
Leaves	0/48h	0/48h	0/48h	0.14
Roots	0/48h	0/48h	50/200 min[f]	83
BRUSSELS SPROUTS (*Brassica oleracea* var. *gemmifera*) (LONG ISLAND)				
Edible part	0/48h	0/48h	0/48h	0.21
Leaves	0/48h	0/48h	0/48h	0.30
Roots	50/3h	50/24h	50/10 min[f]	568
BROCCOLI (*Brassica oleracea* var. *italica*) (GREEN SPROUTING)				
Edible part	0/48h	0/48h	0/48h	0.21
Leaves	0/48h	0/48h	0/48h	0.39
Roots	0/48h	0/48h	50/47 min[f]	181
KALE (*Brassica oleracea* var. *acephala*) (DWARF CURLED SCOTCH)				
Leaves	0/48h	0/48h	0/48h	0.14
Roots	90/24h	0/48h	50/25 min[f]	96
MUSTARD (*Brassica juncea*) (MUSTARD SPINACH)				
Leaves	0/48h	0/48h	0/48h	0.11
KOHLRABI (*Brassica caulorapa*) (EARLY WHITE VIENNA)				
Edible part	0/48h	0/48h	0/48h	0.00
Leaves	0/48h	0/48h	0/48h	0.15
Roots	100/24h	30/48h	50/16 min[f]	165
GARDEN CRESS (*Lepidium sativum*) (CURLED)				
Leaves	0/48h	75/48h	100/2h	0.00[g]
RADISH (*Raphanus sativus*) (EARLY SCARLET GLOBE)				
Edible part	0/48h	0/48h	50/2[f]	0.00[h]

[a] Three grams plant material from all crops, except from roots of cabbages, cauliflower, brussels sprouts, broccoli, kale, and kohlrabi (1.5 grams). [b] Extract representing 15 grams of root material or 100 grams of "greens." [c] D = *Drosophila melanogastor* Meig. [d] M = *Musca domestica* Linn. (C.S.M.A., 1948 strain). [e] h = hours; min = minutes. [f] In addition to contact, also fumigant effect. [g] Two peaks found, with retention times different from that of 2-phenylethylisothiocyanate. [h] Three peaks found with retention times shorter than that of 2-phenylethylisothiocyanate.

133° C., and the detector was operated at 10 volts.

Results and Discussion

Distribution of 2-Phenylethylisothiocyanate in Edible Part of Turnip and Rutabaga. Data obtained with gas chromatographic investigations (Barber-Colman Instrument) of the edible parts of whole turnip and rutabaga, and the peels and pulp thereof, show (Figure 1) that 2-phenylethylisothiocyanate, as represented by peak 3, is the major component in the extracts of all crop parts investigated. The inversion of peak 1*a* as found with rutabaga extract by means of an argon ionization detector at 500 volts indicated very high electron affinity in view of the work done by Lovelock (*6*).

Since 2-phenylethylisothiocyanate apparently reacts with stainless steel, caution is necessary when using this compound. Fluctuations in detector sensitivity to 2-phenylethylisothiocyanate were almost entirely overcome by replacing its stainless steel anode with a

special, gold-plated anode supplied by the Barber-Colman Co.

Quantitative data from both bioassay procedures and gas liquid chromatography (Table I) are in reasonable agreement. Turnip and rutabaga peels (21% of total weight) contained 60 and 47%, respectively, of the total 2-phenylethylisothiocyanate recovered.

Effect of Boiling Crop Material in Water on Persistence of 2-Phenylethylisothiocyanate. Boiling the turnip tissue in water resulted in rapid loss of the toxic constituents (Table II). Nearly all the flies exposed to 3 grams of raw turnip tissue were dead within a 4-hour period. However, tissue boiled for 5 minutes caused only a slight mortality of flies during the same exposure time. No mortalities were registered with tissue boiled for 30 minutes. Mortalities observed with flies held for 24 hours in a bioassay jar not containing turnip tissue were due to lack of food.

2-Phenylethylisothiocyanate was lost with the steam while the water was boiling. Enough toxicants were extracted from the water in which turnip tissue had boiled for 5 minutes to kill nearly all the flies within a 3-hour exposure period. However, no toxic residues could be recovered from water in which turnip peels had been boiled for 30 minutes.

The fractions collected by distilling turnip peel tissue with water were toxic to vinegar flies. The distillate of the first hour contained 250 p.p.m. of 2-phenylethylisothiocyanate, while its concentration in the distillate of the second hour was 147 p.p.m. Fly mortalities were also obtained when insects were exposed to the dry residue of pentane rinsings of the condenser.

Occurrence of Insecticidal Substances in Various Cruciferous Crops. Leaves of turnip and rutabaga investigated contained a toxic material(s) not identical with 2-phenylethylisothiocyanate. This was evidenced by testing two different fractions obtained while eluting the extract from a florisil column. In experiments conducted with root tissue of turnips, 2-phenylethylisothiocyanate was retained on the florosil column during the first 100-ml. pentane wash. However, the chemical could be eluted with a mixture of pentane-ether (6%).

When vinegar flies were exposed to the dry residues of the pentane fractions, representing approximately 100 grams of either turnip or rutabaga leaf material, 40 to 60% of the insects were dead within 24 hours. However, no mortalities were obtained when flies were exposed to the dry residues of the pentane-ether fraction.

With gas liquid chromatography only two peaks—with retention times different from 2-phenylethylisothiocyanate (retention time: 1.1 minutes)—were registered after injection of extracts of either turnip or rutabaga leaves. The retention times of the two peaks were 1.0 and 1.5 minutes.

Typical dosage mortality curves (Figure 2) were obtained after exposure of vinegar flies for 24 hours to the dry residues of aliquots of turnip and rutabaga leaf extracts (pentane fractions).

All cruciferous crops grown in 1962 contained toxicants as evidenced by insect mortalities (Table III) (vinegar flies or houseflies). The insecticidal activities were encountered only with root tissues, with the exception of leaves of garden cress.

With root extracts representing 15 grams of tissue, 50% of the vinegar flies were killed within 10 to 200 minutes. Most effective were roots of brussels sprouts, followed by cabbage (Red Acre), kohlrabi, kale, broccoli, cabbage (Wis. Copenhagen), radish, and cauliflower. Comparable mortalities of vinegar flies were obtained after exposure of the insects to fumes emanating from the dry residue of root extracts.

Injections into the gas chromatograph of nearly all crop extracts resulted in the registering of a peak whose retention time was identical with the retention time of 2-phenylethylisothiocyanate. The concentrations of this peak found in the greens were small and ranged from 0.39 to 0.06 p.p.m. (calculated as 2-phenylethylisothiocyanate), which are sublethal dosages for vinegar flies (*4*). However, within the root tissues investigated, these concentrations ranged from 568 p.p.m. (brussels sprouts) to 83 p.p.m. (cauliflower).

Insecticidal activity, which could not be attributed to 2-phenylethylisothiocyanate, was encountered in leaves of garden cress and the edible parts of radishes. Gas chromatograms from extracts of garden cress leaves showed two peaks with retention times similar to those encountered with leaves of turnip and rutabaga. The injection of extracts of radish root tissue, however, resulted in three peaks whose retention times were shorter than that obtained with 2-phenylethylisothiocyanate.

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