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Diabroticite Beetle Responses to Cucurbitacin Kairomones in *Cucurbita* Hybrids[1]

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Abstract. **Two hybrid cucurbits were produced that combined the genetic production of cucurbitacins as found in the wild bitter gourds, *Cucurbita andreana* Naud and *C. texana* Gray, with the high fruit yields characteristic of the domesticated cultivars of *C. maxima* Duchesne and *C. pepo* L. Both the *C. andreana* × *C. maxima* and *C. texana* × *C. pepo* hybrids produced relatively high yields of fruit with high cucurbitacin content. Both hybrids showed promise as attractants for population estimation of corn rootworm beetles (*Diabrotica undecimpunctata howardi* Barber and *D. virgifera* LeConte) or for use in poisoned baits using methomyl or trichlorfon with the bitter cut fruits or fruit homogenates.**

The fruits of wild species of *Cucurbita* contain substantial amounts of a series of oxygenated tetracyclic triterpenes, the cucurbitacins (10, 14). The cucurbitacins in such wild species as *C. andreana, C. ecuadorensis* Cutl. & Whit., *C. foetidissima* HBK, *C. martinezii* Bailey, *C. okeechobeensis* Bailey, *C. palmata* Wats. *C. palmeri* Bailey, *C. pedatifolia* Bailey, and *C. texana* Gray, are responsible for not only their extremely bitter taste but also for their high toxicity to higher anaimals (7, 17). In contrast, fruits of the edible, domesticated *C. ficifolia* Bouche, *C. maxima* Duchesne, *C. mixta* Pangalo, *C. moschata* Duchesne ex Poir, and *C. pepo* L. either lack or have extremely low concentrations of cucurbitacins.

Most herbivores, including man will not feed on plants containing cucurbitacins which taste bitter in dilutions as low as 1 ppb. It seems likely that these substances were selected through evolution to protect the plants against attack by both invertebrate and vertebrate herbivores. The edible species of *Cucurbita* must have been domesticated through centuries of selection of the least bitter forms by early man. On the other hand, a large group of Diabroticite beetles (Coleoptera, Chrysomelidae, Gallerucinae) have coevolved with *Cucurbita* so that the cucurbitacins have become kairomones, acting as arrestants, and feeding stimulants (4, 6, 9, 12). These beetles including the spotted cucumber beetles *Diabrotica undecimpuncta howardi* Barber, and *D. u. undecimpunctata* Mannerheim, the banded cucumber beetle *D. balteata* LeConte, the western corn rootworm *D. virgifera* LeConte, and the striped cucumber beetle *Acalymma vittata* (Fabricius) and its western relative *A. trivittata* (Mannerheim) are important pests of squash, cucumbers, and muskmelons in North America and *D. undecimpunctata howardi* and *D. virgifera* are severe pests of corn as well. These beetles are compulsive feeders on bitter *Cucurbita* spp. destroying blossoms, leaves, and fruits. They also feed on pure cucurbitacins B, D, E, I, and F-glycoside and can detect amounts of cucurbitacin B as small as 1 ng (12).

Cucurbitacin kairomones are important tools in studying Diabroticite coevolution and behavior (11). They also have possible utility in integrated pest management programs for the cucumber beetles and corn rootworms: (a) as sampling agents for monitoring beetle populations, (b) as ingredients in poison baits, and (c) as trap crops. Large scale field investigations and possible commercial utilization will depend upon the availability of sufficient quantities of bitter *Cucurbita* fruits and of the cucurbitacins. Wild *Cucurbita* spp. do not provide dependable sources of cucurbitacins as they are more difficult to grow and yield less than domesticated spp. and in some cases fruiting is dependent upon photoperiod. Therefore, we have investigated the transfer of cucurbitacin controlling genes from wild species to domesticated species. Suitable germplasm should produce maximum amounts of cucurbitacins and high yields of fruit, together with other factors conferring maximum activity to the *Diabroticites.*

Materials and Methods

Plant materials. The initial crosses consisted of *C. texana* × *C. pepo* cv. Zucchini, and *C. andreana* × *C. maxima* cv. Macre

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(a large fruited form from Peru). Both interspecific hybrids are expected to be fully fertile in the F_1 and succeeding generations (8, 18).

Each entry including the parents was seeded in the greenhouse in early May and transferred to the field (University of Illinois, Agricultural Experiment Station, Vegetable Crops Farm) in late May 1979. Two separate plots of each hybrid and its wild parents were grown. One plot consisted of 6 plants per entry in a single row, with 2 m within and 10 m between rows, together with their parents, 12 other species, and 48 other interspecific hybrids. The other plot consisted of 240 plants per entry, in a single row, 1 m within and 5 m between rows, containing the 2 hybrid spp. and their wild parents.

Determination of cucurbitacins. The cucurbitacins of the parent species and the hybrids were isolated from homogenates of fresh fruit, leaves, blossoms, and roots in chloroform, concentrated to 10:1 (weight/volume); by thin-layer chromatography on silica gel (E. Merck G 254 F or Eastman Chromatosorb) using a solvent of anhydrous ether:hexane:methanol (70:30:5). The cucurbitacins were located by quenching of fluorescence under ultraviolet at 254 nm, by spraying with 5% ferric chloride in ethanol, and by exposing the developed TLC plates in trays containing about 100 adult *D. undecimpunctata* or *D. virgifera.* The beetles were arrested by and fed avidly on microgram quantities of the cucurbitacins (3, 4, 6, 12) leaving clear spots on the chromatographic plates wherever the cucurbitacins were present (Fig. 1). Identification of specific cucurbitacins (Cu) was made by R_f compared to pure cucurbitacins and by elution of the spots followed by mass spectrometry (1). Quantitative determinations of the individual cucurbitacins were made on methanol eluates of the spots on the TLC plates, using ultraviolet spectrophotometry at 210 nm. Standard curves from individual pure Cu B, Cu E, and Cu E glycoside were prepared in absolute spectrograde methanol. The limit of detection of the cucurbitacins was 1 ppm.

Results and Discussion

Plant characteristics. The *C. texana* x *C. pepo* hybrid produced semibush plants with leaves, fruits, and blossoms resembling *C. pepo* cv. Zucchini (Fig. 2). The 6-plant plots produced 98 fruits with an average weight of 0.73 kg. The *C. texana* parent produced small fruits, averaging 0.10 kg, about as early as the hybrid but in much lower quantity.

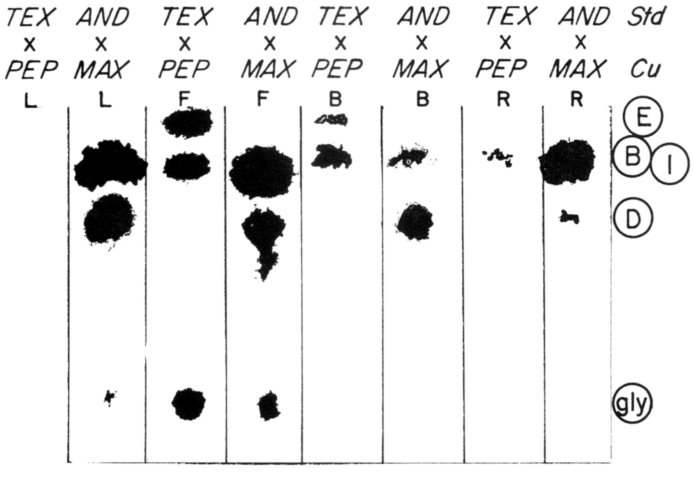

Fig. 1. Profiles of thin-layer chromatograms prepared from standard chloroform extracts of squash leaves (L), fruits (F), blossoms (B), and roots (R). Cucurbitacin-containing areas eaten from the plates by *Diabrotica undecimpunctata howardi* are shown in black. TEX x PEPO = *C. texana* x *C. pepo* hybrid and AND x MAX = *C. andreana* x *C. maxima* hybrid.

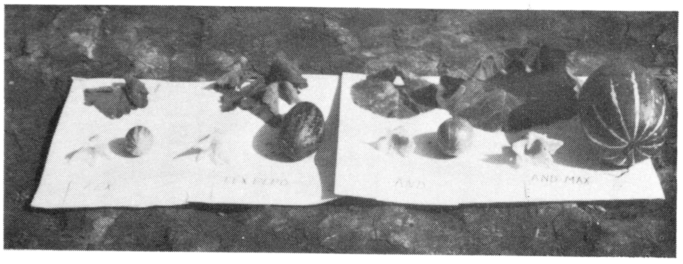

Fig. 2. Leaves, fruits, and blossoms (from left to right) of *C. texana, C. texana* x *C. pepo* hybrid, *C. andreana,* and *C. andreana* x *C. maxima* hybrid.

The *C. andreana* x *C. maxima* hybrid produced long-vined plants with leaves, fruits, and blossoms resembling *C. maxima* (Fig. 2). The 6-plant plot produced 84 fruits with an average weight of 3.90 kg. The *C. andreana* parent produced fruits later than its hybrid, averaging 0.50 kg, and in lower quantity. Yields of the domestic parents were not measured.

Cucurbitacin analysis. Spectrometric analysis of the chloroform extracts of leaves, fruits, roots, and blossoms of the *Cucurbita* spp. are shown in Table 1. These values represent the means of 3-5 replicate determinations of the individual components taken from the TLC plates unless indicated otherwise. In general the samples extracted were composites of 5-10 fruits, leaves, and roots, and about 20 blossoms.

It is evident from the Diabroticite feeding on TLC plates (Fig. 1) and from R_f values compared to standard cucurbitacins that *C. andreana* and its *C. andreana* x *C. maxima* hybrid contained principally Cu B and Cu D and *C. texana* and its *C. texana* x *C. pepo* hybrid contained principally Cu E, largely as the glycoside (elaterinide) with some free Cu E and Cu I. These conclusions were confirmed by the immediate blue-violet color produced by the diosphenols Cu E and Cu I after spraying with ethanolic ferric chloride, and by high resolution mass spectrometry of the individual spots on the TLC plates which conclusively demonstrated Cu B, Cu D, Cu E, and Cu E glycoside (unpublished data).Very high concentrations of Cu B and

Table 1. Cucurbitacin content of certain squash and hybrids.

Species	Organ	Cucurbitacin content (μg per g fresh wt) B	D	E	I	E-glycoside
C. andreana	leaf	0.15	0.12			
	fruit	2.78	0.42			
	blossom	0.19	0.12			
	root	0.58	0.51			
C. andreana x *C. maxima*	leaf	0.56	0.23			
	fruit	1.17	0.09			
	blossom	0.16	0.17			
	root	0.26	0.09			
C. texana	leaf			trace	trace	trace
	fruit			0.07	0.36	0.75
	blossom			0.24	0.15	0.50
	root			0.18	0.08	0.39
C. texana x *C. pepo*	leaf			trace	trace	trace
	fruit			0.23	0.09	0.16
	blossom			0.10	0.14	trace
	root			0.09	0.04	trace
C. maxima	no cucurbitacins detected in fruit and root					
C. pepo	no cucurbitacins detected in fruit and root					

Table 2. Numbers of *Diabrotica undecimpunctata* (SCR) and *Diabrotica virgifera* (WCR) attracted to cucurbit blossoms and crushed leaves.[z]

Species	Diabrotica beetle	No. of beetles			
		Blossoms		Leaves	
		Aug. 28	Sept. 6	Aug. 7	Aug. 28
C. andreana	SCR	19 ± 9	11 ± 5	7 ± 6	42 ± 30
	WCR	3 ± 3	1 ± 1	5 ± 4	21 ± 18
C. andreana x *C. maxima*	SCR	23 ± 6	19 ± 7	22 ± 12	18 ± 18
	WCR	8 ± 5	1 ± 1	38 ± 28	10 ± 10
C. texana	SCR	8 ± 4	4 ± 2	3 ± 2	13 ± 8
	WCR	5 ± 3	1 ± 1	3 ± 3	1 ± 3
C. texana x *C. pepo*	SCR	7 ± 4	5 ± 2	11 ± 8	17 ± 13
	WCR	9 ± 4	6 ± 3	21 ± 8	3 ± 3

[z]Mean values of 20 ± SD.

Cu D, about 0.3% were found in *C. andreana* fruit (Table 1). *C. maxima,* the non-bitter parent, contained only trace amounts of cucurbitacins not detectable by TLC or ultraviolet spectrophotometry and the *C. andreana* x *C. maxima* hybrid generally had concentrations intermediate between the 2 parents, except for a clear cut enhancement in the leaves, that was anticipated from the field experiments on beetle feeding (Table 2). Thus, the cucurbitacin content of the bitter hybrid fruits represents an example of incomplete dominance.

For *C. texana* most of the cucurbitacin content in fruit and leaves was present as Cu E-glycoside (elaterinide). The *C. texana* x *C. pepo* hybrid had lowered conjugating capacity (Fig. 1 and Table 1), and substantially greater amounts of free Cu E and Cu I were present than in *C. texana.* The other parent *C. pepo* was essentially devoid of cucurbitacins, not detectable by TLC or ultraviolet spectrophotometry.

At least 5 independent genes are said to regulate the biosynthesis of cucurbitacins: (a) a gene *Bi* that regulates synthesis in seedlings, (b) a gene su^{Bi} that suppresses synthesis of cucurbitacins in fruits, (c) a gene that controls the quantity of cucurbitacins, (d) a gene that governs the chemical nature of the cucurbitacin formed, and (e) a gene Mo^{Bi} that determines whether the cucurbitacin exists as a free aglycone or as a glycoside. Bitterness in Cucurbitaceae was originally thought to be regulated by a single dominant gene *Bi* (15) and plants with non-bitter seedlings *bi bi* did not synthesize cucurbitacins. However, later evidence (15) showed that non-bitter fruits could develop from bitter seedlings and it was suggested (2) that a single recessive suppression gene su^{bi} prevented bitterness in fruits, which could be expressed by the dominant allele su^{bi}. Organospecific genes appear to control the qualitative and quantitative formation of cucurbitacins in leaves, fruits, blossoms, and roots (14). A single modifier gene Mo^{bi} that acts only in the presence of *Bi* and Su^{bi} alleles apparently controls the quantity of Cu E-glycoside (elaterinide) formation in bitter fruit (2).

Attraction of Diabroticites to Cucurbita. C. andreana and *C. texana* were selected on the basis of previous study as especially attractive to *D. undecimpunctata howardi* and to *D. virgifera* (9). Cultivated plants of these species suffered severe beetle damage to leaves and fruits, and blossoms of *C. andreana* were often so severely eaten as to prevent fruit set. In contrast, little beetle feeding occurred on *C. maxima* or *C. pepo.* The numbers of these 2 species of beetles attracted to and feeding on blossoms, crushed leaves, or cut fruits of the 2 bitter squash and their hybrids are shown in Tables 2 and 3. The 2 hybrids are clearly very attractive to the beetle and are approximately as effective as the parental bitter species (see also Table 6).

Bitter squash fruit baits for Diabroticites. Bitter squash containing high concentrations of cucurbitacins has been recognized as exceptionally attractive to Diabroticites since Contardi (5) showed that the beetle *Diabrotica speciosa* Germ. preferred to feed on the bitter gourd *C. andreana* and ignored the sweet squash *C. maxima.* When equal numbers of split squash fruits were sampled 99% of the beetles were collected from the bitter squash. Sharma and Hall (16) found that southern corn rootworm beetles *D. undecimpunctata howardi* (SCR) were attracted to cut *C. foetidissima* fruits, high in cucurbitacins, about 15-fold more abundantly than to *C. pepo* fruits low in cucurbitacins. More recently, Howe et al. (9) showed the western corn rootworm beetles, *D. virgifera* (WCR) were attracted to cut fruits of *Cucurbita* in the following mean numbers: *C. maxima* 0.3, *C. pepo* 0.5, *C. andreana* 13.3 and *C. texana* 14.0.

Table 3. Comparison of cut fruits vs. homogenates of *Cucurbita* hybrids as attractants for *D. undecimpuncta.*

Time exposed (hr)	Avg number of beetles per half fruit[z]			
	C. andreana x *C. maxima*		*C. texana* x *C. pepo*	
	Cut	Homogenized	Cut	Homogenized
0.5	3 ± 2	86 ± 115	2 ± 1	159 ± 84
1	6 ± 4	179 ± 153	6 ± 5	220 ± 143
2	16 ± 12	266 ± 278	13 ± 11	381 ± 151
4	213 ± 160	>500	88 ± 38	>500
30	460 ± 218	ca 1000	251 ± 154	ca 1000

[z]Avg of 4 replicates of each cross.

Table 4. Numbers of *Diabrotica undecimpunctata* (SCR) and *Diabrotica virgifera* (WCR) attracted to and feeding on cut cucurbit fruits.

Time exposed (hr)	Avg number of beetles per half fruit[z]			
	C. texana x *C. pepo*		*C. andreana* x *C. maxima*	
	SCR	WCR	SCR	WCR
0.25	10 ± 5	3 ± 2	4 ± 3	2 ± 3
0.5	16 ± 8	6 ± 6	6 ± 4	3 ± 3
1	26 ± 11	19 ± 18	16 ± 10	8 ± 7
2	45 ± 18	17 ± 11	21 ± 8	10 ± 9
4	101 ± 16	55 ± 31	99 ± 26	61 ± 29

[z]Avg of 10 replicates of each cross ± SD.

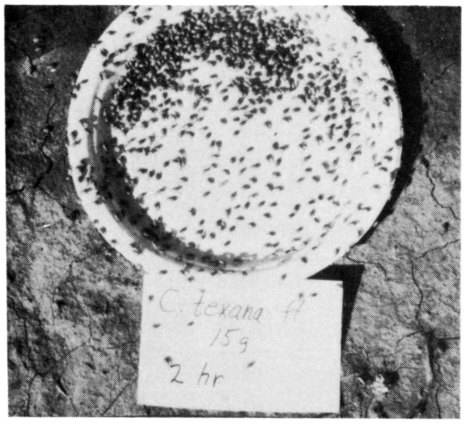

Fig. 3. Diabrocite beetles killed within 2 hr after feeding on 15 g of *C. texana* homogenate, containing 0.01 g methomyl insecticide.

Fig. 4. Diabroticite beetles killed after 5 days by feeding on cut fruit of *C. texana* x *C. pepo* hybrid dusted with 0.1 g methomyl insecticide.

As reported earlier (13) homogenates of the fruit of *C. texana* and *C. andreana* at 100 g in 500 ml of water, used at ca. 15 g in a 10 in (25 cm) paper pie plate attracted hundreds of *D. undecimpunctata, D. virgifera,* and *A. vittata* within a few hours, the beetles eating avidly until the fruit was entirely consumed. A typical experiment involving a population of *D. undecimpunctata* is shown in Table 3, where fruits of *C. andreana* x *C. maxima* and *C. texana* x *C. pepo* were cut in halves longitudinally and one-half exposed as a homogenate in 100 ml of water and the other as the cut fruit. The homogenate attracted the beetles much more rapidly than the cut fruits but after 4 hours both preparations attracted hundreds of beetles. The *C. andreana* x *C. maxima* fruit was somewhat more attractive than the *C. texana* x *C. pepo* fruit. In another experiment using cut fruits, similar results were obtained for *D. undecimpunctata* and *D. virgifera* attraction (Table 4).

Table 5. Comparative effectiveness of baits of cut *C. texana* x *C. pepo* fruit treated with ca 0.1 g methomyl or trichlorfon.

Time after treatment (hr)	insecticide treatment	Mean number of Diabroticites attracted	
		SCR	WCR
5	Methomyl	61 ± 34[z]	17 ± 16[z]
	Trichlorfon	24 ± 8[z]	3 ± 1[z]
	Unpoisoned	16[y]	3[y]
10	Methomyl	94 ± 43	20 ± 20
	Trichlorfon	46 ± 18	7 ± 4
	Unpoisoned	39	20
22	Methomyl	122 ± 63	22 ± 17
	Trichlorfon	65 ± 22	7 ± 14
	Unpoisoned	62	20
48	Methomyl	241 ± 67	40 ± 37
	Trichlorfon	215 ± 103	19 ± 14
	Unpoisoned	24	15
72	Methomyl	329 ± 92	54 ± 47
	Trichlorfon	361 ± 180	24 ± 20
	Unpoisoned	15	16
96	Methomyl	455 ± 96	63 ± 6
	Trichlorfon	481 ± 178	43 ± 28
	Unpoisoned	10	9

[z]Avg of 5 replications ± SD, beetles dead.
[y]Avg of 2 replicates, beetles alive.

Poisoned baits. The effectiveness of the bitter squash homgenates was greatly improved by adding 0.01 to 0.1% (weight/volume) of the water soluble insecticides methomyl (water solubility 5.8 g per 100 ml at 25°C) or trichlorfon (water solubility 15.4 g per 100 ml at 25°). These poisoned baits killed beetles feeding on the baits within 2-5 min and prevented them from eating appreciable quantities so that the baits remained effective for several days (see Fig. 3).

Using a modified "salt shaker," technical methomyl or trichlorfon was sprinkled on the cut halves of *C. texana* x *C. pepo* fruits at about 0.1 g per 100-200 g half fruit. Ten treated fruit halves were placed 10 m apart in rows at the edge of a large squash plot. The results in Table 5 show that both *D. undecimpunctata* and *D. virgifera* were attracted to and killed by the poison baits in substantial numbers and that the baits remained highly effective for more than 7 days (see Fig. 4). Although the methomyl-treated baits were initially somewhat more effective than the trichlorfon baits, there was no significant difference after 2 days.

To determine if the hybrid fruits were as effective as their wild parents, a similar experiment was repeated with *C. andreana* fruits (average weight 272 g), *C. texana* (96 g), *C. andreana* x *C. maxima* (316 g), and *C. texana* x *C. pepo* (228 g), using ca 0.1 g methomyl per half fruit. As shown in Table 6, there was no significant difference in the effectiveness of any of the baited fruits and they killed large numbers of beetles despite a heavy rain that fell the evening after the experiment was begun. The dead beetles were so numerous that they were not separated as to species after the first day.

Attraction of Diabroticites in corn. The cut fruits of *C. texana* x *C. pepo* treated with ca 0.1 g of methomyl or

Table 6. Attraction of Diabroticites to cut fruits of *Cucurbita* parents and hybrids treated with ca 0.1 g methomyl.

Time exposure (hr)	Avg number of dead beetles per cut fruit[z]			
	C. andreana	*C. andreana* x *C. maxima*	*C. texana*	*C. texana* x *C. pepo*
24	17 ± 12 (70% SCR)[y]	28 ± 26 (80% SCR)[y]	11 ± 8 (73% SCR)[y]	20 ± 9 (81% SCR)[y]
48	325 ± 92	342 ± 179	259 ± 100	330 ± 86
96	474 ± 136	496 ± 238	282 ± 150	513 ± 97

[z]Avg of 10 replicates ± SD.
[y]By observation.

trichlorfon were very effective in killing Diabroticite beetles in a field of sweet corn (*Zea mays* L.) even after 3 heavy rains totalling 5 cm. Five cut fruit halves placed 5 m apart killed the following mean numbers of beetles after 5 days: methomyl (SCR 227 ± 95, WCR 19 ± 13) and trichlorfon (SCR 287 ± 159, WCR 40 ± 39).

It is evident from these and many other experiments performed with bitter fruits as poison baits, that these are effective in killing large numbers of Diabroticite beetles. Such traps have possible utility in protecting vegetable gardens from beetle attack and in monitoring beetle populations in corn fields. Various traps have been employed using homogenates of bitter fruits poured on vermiculite, sponges, or paper; and cut fruits placed in buckets and other containers.

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Overcoming Self-incompatibility in *Raphanus sativus* L. with High Temperature[1]

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Abstract. **Self-incompatibility of *Raphanus sativus* L. was partially overcome by exposing plants to temperature of 30 to 50°C. The most effective treatment was 50°C for 25 minutes. Scanning electron microscopical (SEM) observation of pollen tubes on the papillae surface have shown that exposure of the gynoecium to 50°C for 25 minutes resulted in pollen tube growth following self-pollination that resembled that of cross-pollination. Openings of papillae and detached pollen grains and tubes were found as the result of successful pollen tube penetration of papillae. Fluorescence microscopical (FM) observation served to confirm these observations made by SEM. However, incompatible pollen failed to germinate although pollen grains were attached to the papillae by aid of their waxy surface substances.**

Most of *Raphanus sativus* cultivars are self-incompatible, and the response is generally known to be a sporophytic type (5, 30, 31). In order to breed pure lines or to retain parent lines for F_1 seed production, overcoming self-incompatibility is an important problem. The methods of overcoming self-incompatibility such as hormone application (8, 9, 18), temperature treatments (1, 3, 11, 12, 15, 25, 32), mentor pollen application (7, 14, 26, 28, 29), bud or old flower pollination (2, 27), placental, ovarian, or test tube pollination (4, 13, 20) and a mechanical or electric method (22, 23, 24) differ depending on plant genera. The self-incompatibility of *R. sativus* may be partially overcome by either bud pollination or CO_2 gas treatment (19), bud pollination has been used on a commercial basis. Recently, the effect of various high temperature treatments or mentor pollen application to promote pollen germination on incompatible stigma has been reported (30, 31).

This paper presents the result of studies conducted with high temperature treatments for overcoming the self-incompatibility with observations made during the course of these investigations with SEM and FM on the interaction between self-incompatible pollen and stigma.

Materials and Methods

Selections of self-incompatible plants from 'Honbashi-taibyo-Minowase' (H-Mino) and 'Minowase' (Mino) radish were used.

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