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Short communication

Hypersensitivity reaction of *Brassica nigra* L. (Cruciferae) kills eggs of *Pieris* butterflies (Lepidoptera: Pieridae)

A.M. Shapiro [1] **and J.E. DeVay** [2]

[1] Department of Zoology, University of California, Davis, CA 95616, USA
[2] Department of Plant Pathology, University of California, Davis, CA 95616, USA

Summary. Some individual plants of the mustard *Brassica nigra* in lowland California kill eggs of the Crucifer-specialist herbivores *Pieris rapae* and *P. napi* by producing a necrotic zone at the base of the egg, thereby apparently desiccating it. This is a typical hypersensitivity reaction, but to an atypical stimulus. The eggs can be "rescued" by maintaining them in a saturated atmosphere. Attempts to demonstrate a bacterial or fungal agent associated with the reaction were unsuccessful.

Key words: Hypersensitivity – *Pieris* – *Brassica* – Herbivory – Defense

One type of defense used by higher plants against attacks by pathogens is the hypersensitivity response (HR) described by Stakman (1915). In HR the cytoplasm of cells at the site of attack becomes disorganized and granulated; there is rapid cell collapse and death, effectively containing the attackers by isolating them from healthy tissue. HR has been reported in both monocots and dicots and in response to fungal, viral and bacterial invasions (Klement and Goodman 1968; Wood 1982). We have been unable to find any report of HR in response to insect attack.

Black Mustard, *Brassica nigra* L. (Cruciferae), is a common, naturalized, weedy annual or winter annual at low elevations in California, USA. One of its commonest herbivores is *Pieris rapae* L. (Lepidoptera: Pieridae), also naturalized from Europe. Since 1981 one of us (AMS) has repeatedly observed that eggs of *P. rapae* laid on certain individuals of *B. nigra* in central California invariably discolor, shrivel and die as a necrotic zone appears around the base of the egg. The plants on which this occurs are otherwise indistinguishable from the general *B. nigra* population. The presence of such "killer" plants was first detected at Suisun City, Solano County, where they appear to be common, and has subsequently been confirmed at Vacaville, Solano County, and Davis and West Sacramento, Yolo County, where they appear to be rare. We have used material from about 25 plants.

Egg-killing is easily reproducible in the laboratory. Either all leaves or no leaves of a given plant react to *P. rapae* eggs in this manner. Both rosette- and cauline foliage react. We have obtained reactions from cuttings of stems maintained in water, both prior and subsequent to rooting; from isolated leaves maintained on Water-pics; and from transplanted wild rosettes in pots. We have not studied transmission of the "killer" trait by seed. The reaction is most pronounced if the foliage is in full sunshine or at high temperatures (above 30° C for at least a few h/day) and may not occur under fluorescent illumination at continuous 24° C. We have observed HR afield as late as late October, however, at Suisun City.

Pieris napi L. *sensu lato* is a native, non-weedy species in California which occurs in moist riparian habitats in foothill canyons and the lower montane zone and has little or no contact with *B. nigra*. Three northern California subspecies of *P. napi* (*microstriata* Comstock of the Inner Coast Ranges and Sierra foothills, *venosa* Scudder of the Outer Coast Ranges south of San Francisco Bay, and *marginalis* Scudder of the north coast) have been tested with "killer" *B. nigra*, and all trigger HR as effectively as *P. rapae*.

We have carried out 16 split-brood experiments, in which individual *P. rapae* or *P. napi* oviposited on alternate days on cuttings of "killer" or "non-killer" *B. nigra* or on "killer" *B. nigra* and other Crucifers. Egg-killing is an all-or none phenomenon; in all trials either all eggs or none were killed. We have encountered three individual female *P. rapae* whose eggs failed to elicit HR on "killer" plants, even when the eggs were incubated in sunshine. Neither the females nor the eggs were phenotypically recognizable. No female has switched between eliciting and not eliciting HR. All five *P. napi* tested triggered HR.

The necrotic zone (Fig. 1) develops within 24 h after oviposition at 32° C. By 36 h the egg shows a characteristic orange-ochre color and initial signs of collapse, and by 72 h has shrivelled entirely, and often fallen off. Because *Pieris* eggs fail to hatch if the plant on which they were laid is allowed to desiccate, but can be "rescued" by maintaining them in a saturated atmosphere (AMS, unpublished observation), we tried placing eggs of both *Pieris* in or near 100% RH as soon as the leaf began to necrose visibly. From 80–100% of eggs placed in closed Petri dishes with abundant condensation hatched unless mold supervened, whether the eggs were detached or allowed to remain on the substrate. Control eggs, attached or detached, never hatched under ambient humidity (generally about 30%). We infer that the eggs are killed by desiccation provoked by the necrosis of the plant tissue around their bases. This implies that *Pieris* eggs are in water equilibrium with their substrate, such that desiccation of the substrate draws water out of the egg. We have found no literature on the physiology of water balance in Pierid eggs.

Offprint requests to: A.M. Shapiro

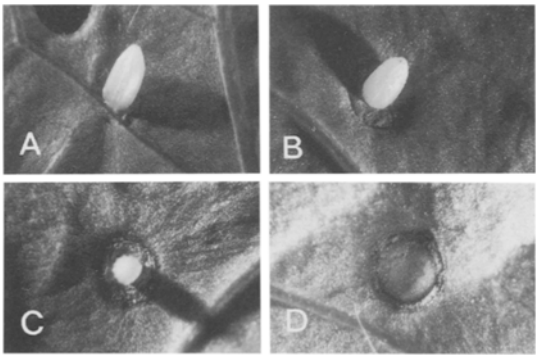

Fig. 1 A–D. *Pieris rapae* eggs on *Brassica nigra.* **A** 8-h-old egg with beginning of necrosis barely visible at base. **B** and **C** two 36-h-old eggs with pronounced necrotic zones. **D** necrotic zone after dropping of shrivelled egg at 80 h

Since the HR observed is quite normal as a response to plant pathogens, we tested the possibility that a pathogen was introduced at the time of oviposition by inoculating isolations from the necrosing tissue at egg bases directly onto potato-dextrose and nutrient agar media. Saprophytic, non-invasive organisms of the genera *Aspergillus, Penicillium,* and *Bacillus* were recovered. None of these is likely as an inducer of HR. Viruses have not been excluded. Given the diversity of sources of *Pieris* used (*P. rapae* from Yolo, Solano, Sacramento and Nevada Counties and *P. napi* from Solano, Humboldt, San Mateo and Nevada Counties), unless the putative pathogen is ubiquitous on the ovipositors of *Pieris,* it is more parsimonious to suggest that some surface component of *Pieris* eggs is capable of triggering HR in susceptible individuals. We suspect a component of the egg cement, because eggs removed on a moist brush and placed on the surfaces of "killer" leaves rarely induce necrosis. Thus, chorion components are unlikely to be involved. The chemistry of *Pieris* egg cement appears undocumented; Rothschild and Schoonhoven (1977) suggested that an oviposition-deterrent pheromone might be deposited with eggs of *P. brassicae* L.

Egg-killing is a unique form of defense against herbivory. It is highly specific and seemingly cost-effective, since extensive tissue damage can be avoided at the price of very localized tissue necrosis. Whether it represents an evolved response to herbivory or a fortuitous response to some substance present on eggs remains to be seen. It has not been observed in *Brassica campestris* L., *B. oleracea* L., *B. geniculata* (Desf.) J. Ball, or *B. kaber* (DC.) Wheeler, all of which are *P. rapae* hosts and all of which have been tested repeatedly in the laboratory over 5 yr, or in any other Crucifer, and it is present only in certain individuals of *B. nigra.* We would not be surprised if both egg-killing and the ability to trigger it were polymorphic in populations of *B. nigra* and *P. rapae* respectively, just as "gene-for-gene" pathogen-resistance systems are found in plants. If a genetic basis for egg-killing can be demonstrated, it could prove useful in biological control of *Pieris* as a pest on cole crops.

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