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Touch-sensitive glandular trichomes: a mode of defence against herbivorous arthropods in the Carboniferous

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ABSTRACT

We present evidence that the capitate glandular trichomes of *Blanzyopteris praedentata*, a lianescent seed fern from the Upper Carboniferous of France, possessed a specialized, touch-sensitive mechanism that triggered the opening of the secretory cell by contact. The trichomes are interpreted as functionally similar to those of some modern flowering plants, which release a sticky exudate when touched and ruptured that functions to disable plant-feeding arthropods.

Keywords: defence mechanism, glandular trichome, herbivory, seed fern, Upper Carboniferous.

Arthropods have used plants as a food source for at least 400 million years. Evidence for ancient herbivory appears in the form of coprolites containing partially digested plant materials from the Siluro-Devonian (Edwards *et al.*, 1995). Furthermore, bite marks on leaves (e.g. Scott and Taylor, 1983), stylet probes (Labandeira and Phillips, 1996), tissue disruption (e.g. Cichan and Taylor, 1982) and wound-callus formation in response to animal attacks (Banks, 1981) are well-documented examples from the Devonian and Carboniferous, which demonstrate the complex relationships between plants and plant-feeding arthropods in the Palaeozoic. One aspect that has scarcely been addressed concerns the mode of defence used by ancient plants in response to herbivory. In extant plants, physical (e.g. trichomes, thorns, prickles) and chemical (e.g. repelling secondary metabolites) defence systems discourage potential feeders (Levin, 1973; Howe and Westley, 1988; Bennett and Wallsgrove, 1994). Gathering information about the mechanisms used to

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defend against herbivory in ancient plants is a challenge because the fossil record is incomplete. Nevertheless, studies of the evolution of herbivory and herbivores based on fossil organisms represent the only method of tracing this ancient 'arms race' that serves to define interrelationships in the ecosystems through time. Here, we present compelling evidence that *Blanzyopteris praedentata* (Gothan) Krings et Kerp, a tendril-climbing Late Carboniferous seed fern (pteridosperm), possessed touch-sensitive glandular trichomes, which were effective in discouraging herbivorous arthropods.

Compression specimens of *Blanzyopteris praedentata* come from several open-cast mines, situated along the southeastern margin of the Blanzy-Montceau Basin near the city of Montceau-les-Mines (Massif Central, France). The coal-bearing strata have been dated as late Stephanian (~290 million years B.P.) (Branchet, 1983). The glandular trichomes were studied from cuticles prepared according to procedures outlined in Kerp (1990); permanent slides are deposited in the Münster palaeobotanical collection (collection numbers PBO Np.1 to PBO Np.764).

Blanzyopteris praedentata (Fig. 1a) possessed numerous glandular trichomes on the lower surfaces of all foliage parts and on the tendrils. The trichomes are between 0.5 and 1.1 mm long and consist of a uniseriate stalk of 3–10 cells and usually one enlarged apical secretory cell. The secretory cell bears a multicellular filament formed of small cells (Fig. 1b). This filament, however, is missing in most samples studied (Fig. 1c,d), and the secretory cells are always open at the point where the filament was once attached (Fig. 1d). We hypothesize that the secretory cell opened as a result of a stimulus to the filament, and that the filament functioned as a trigger in the opening mechanism, activated by arthropods that moved around on the plant.

Functionally similar, touch-sensitive glandular trichomes occur in several living flowering plants (e.g. Cucurbitaceae, Solanaceae). In *Sicana odorifera* (Vellozo) Naudin, for example, a member of the Cucurbitaceae, the touch-sensitive glandular trichomes are up to 0.2 mm long and consist of a uniseriate stalk of 3–6 cells and a two-celled head, including an enlarged, secretion-filled cell on top of which a second, small and papillate cell is present (Fig. 1e). When touched and rupturd by an arthropod (e.g. an aphid), touch-sensitive glandular trichomes rapidly release a sticky exudate, which precipitates on the arthropod's legs (Levin, 1973). The exudate may eventually cement the arthropod to the leaf surface. On the other hand, the gradual accumulation of exudate may also reduce the ability of the arthropod to securely grip the leaf surface by encasing the distal parts (i.e. tarsus and pretarsi) of the legs (Fig. 2a,b); arthropods disabled by ineffective legs become easily dislodged from the leaf by air currents over the phylloplane. We hypothesize that the glandular trichomes of *Blanzyopteris praedentata* (Fig. 1b–d) functioned in a similar way to that described above by discouraging herbivorous arthropods on this Carboniferous plant. Species of the Palaeodictyopteroidea, a group of piercing-and-sucking insects widespread in the Late Carboniferous (Labandeira and Phillips, 1996), represent excellent candidates for encountering the defence involving touch-sensitive glandular trichomes of *Blanzyopteris praedentata*. The accumulation of exudate on the legs would significantly inhibit their ability to maintain contact with the leaf. Moreover, to feed by extracting liquids, palaeodictyopteroids had to pierce the epidermis and underlying tissues of the plant with their (long) proboscides; here leverage on the phylloplane was crucial for successful penetration of the plant surface. Such leverage is generated through the animal's firm grasp to the leaf surface while piercing and thus, without effective legs, the plant surface becomes impenetrable and the animal subsequently starves.

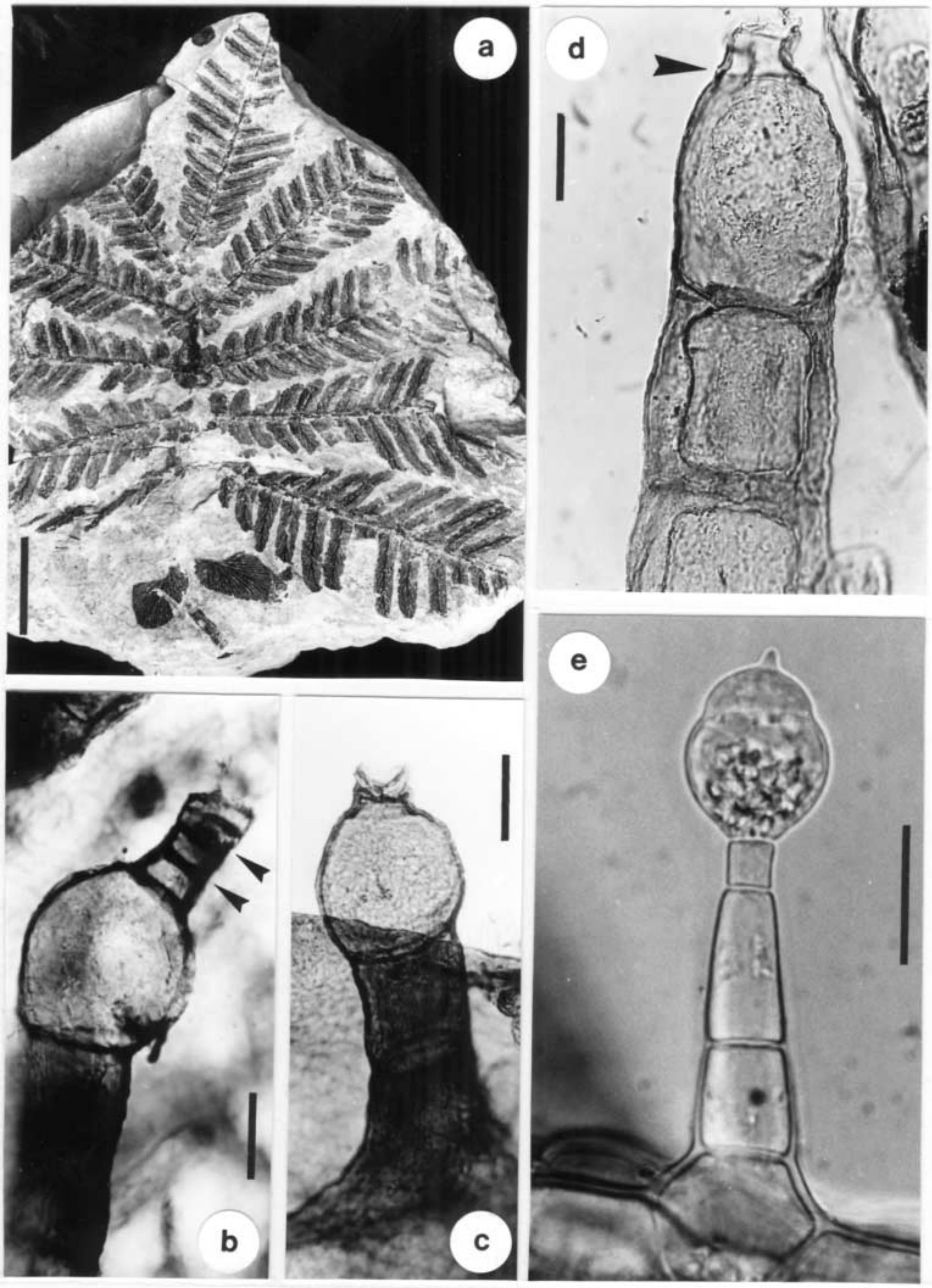

Fig. 1. (a) *Blanzyopteris praedentata* frond; bar = 4 cm. (b) Glandular trichome of *B. praedentata* with enlarged secretory cell that bears a filament of small cells (arrows); bar = 100 μm. (c) As for (b), but with the filament broken off; bar = 110 μm. (d) Empty secretory cell, open where the filament was attached (arrow); bar = 30 μm. (e) Touch-sensitive glandular trichome of *Sicana odorifera*; bar = 35 μm.

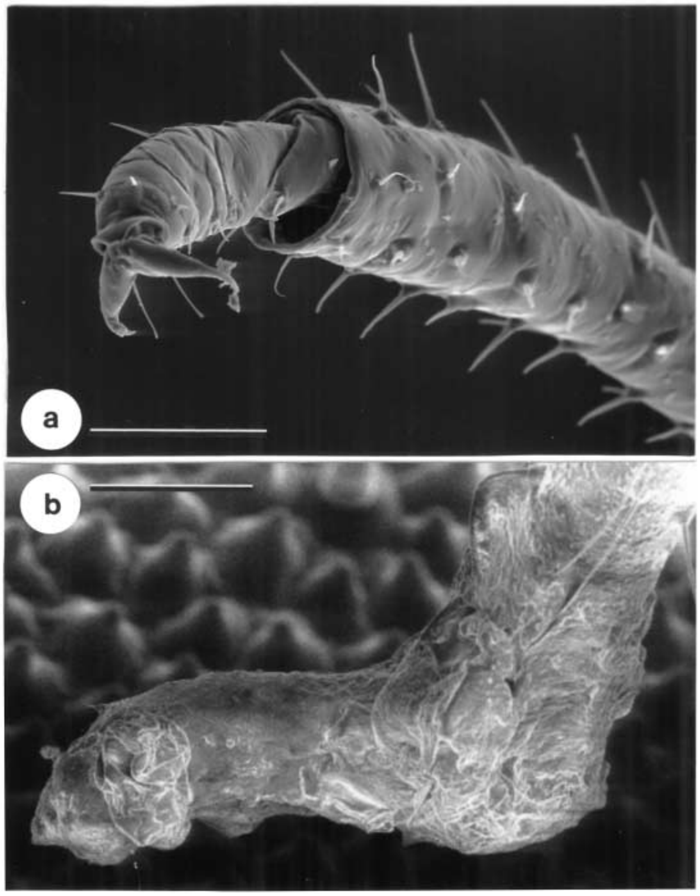

Fig. 2. (a) Tarsus and pretarsi of an aphid (*Aphis nerii*) leg; bar = 50 μm. (b) Distal parts of an aphid leg, which encountered the glandular hairs of *S. odorifera*. The tarsus and pretarsi are encased with exudate; bar = 50 μm.

Trichomes on fossil plants have been noted in some of the earliest studies and used in systematic considerations (cf. Kerp, 1990). Others have also used plant trichomes as a proxy record of habitat conditions (e.g. Barthel, 1962). The touch-sensitive glandular trichomes on the foliar parts and tendrils of *Blanzyopteris praedentata* offer still another interpretation, which strongly implies a specific interaction with arthropods that inhabited the Late Carboniferous coal swamp ecosystems. *Blanzyopteris praedentata* was a liana that utilized compound tendrils terminating in adhesive pads to attach to a mechanical support (Krings and Kerp, 1999). The species displays various stem and foliage modifications and anatomical adaptations to the special biomechanical and physiological requirements of the liana-like growth. The defence system against herbivory involving touch-sensitive glandular trichomes indicates that this liana was also well-adapted to the environmental parameters to which it was exposed. *Blanzyopteris praedentata* appears to have been a 'well-armed' Carboniferous plant, which had the capacity to face the abundance of plant-feeding arthropods at the end of the Palaeozoic (cf. Labandeira and Sepkoski, 1993). Hegarty *et al.* (1991) hypothesized that lianas/vines may have evolved higher levels of protection from herbivores than those of trees. This assumption is based on a higher surface-to-volume ratio than in trees because of a greater leaf biomass and leaf area when plotted against total biomass. In addition, evergreen climbers in (sub-)tropical climates may possess a larger proportion of young stems and leaves, which may require special protection during their development. The specialized defence system against herbivory involving touch-sensitive glandular trichomes is well-correlated with the liana-like life-form. Among the other seed ferns from the same ecosystem are various secretory structures (e.g. glandular trichomes, secretory cavities), which are abundant in scrambling/climbing forms but rarely found in tree-like taxa (Krings, 2000). This suggests a relationship to special demands unique to the protection of climbing plants. Such special demands might have been fulfilled by the various structures secreting secondary metabolites with repelling properties.

The comments offered here on the glandular trichomes of *Blanzyopteris praedentata* are formulated on a function-based ecological analogy between this fossil and living flowering plants (e.g. Arens, 1997). Our hypothesis about the effectiveness of the trichomes of *Blanzyopteris praedentata* is based on structural correspondences with trichomes of modern plants for which the functions are known. This analogy, however, is limited to rather general interpretations, and does not allow more than speculation on those aspects of the interaction that have been clarified statistically or experimentally in modern plants. For example, we do not know the actual effectiveness of the defence system involving touch-sensitive glandular trichomes in *Blanzyopteris praedentata*. In research on extant plant–herbivore interactions, the effectiveness of a defence mechanism can be analysed statistically; the damage caused by herbivores is used as a simple but reliable proxy record (e.g. Karban and Baldwin, 1997, and literature cited therein; Schappert and Shore, 1999; Valverde *et al.*, 2001). Damaged parts of *Blanzyopteris praedentata* have not been discovered to date, which could indicate that the defence system was highly effective. However, it is interesting to note that rather few records of damage by herbivores in Carboniferous seed fern foliage exist. Perhaps the seed ferns were generally 'well-armed' against herbivores and thus only infrequently visited.

In attempting to estimate the damage caused by herbivores in fossil plants, it is important to consider two parameters. First, the manner in which the plant is preserved. Since most Carboniferous seed fern taxa, including *Blanzyopteris praedentata*, are preserved as impressions (i.e. imprints in the rock matrix with no organic material preserved) or compressions (i.e. thin carbonized films with no internal structure preserved), it remains almost impossible to recognize damage due to herbivore activity, with the possible exception of bite marks. The second parameter is the type of damage. During the Carboniferous, an important group of plant-feeding arthropods (i.e. the Palaeodictyopteroidea) possessed piercing-and-sucking mouthpart morphology (Labandeira and Sepkoski, 1993; Labandeira and Phillips, 1996). The stylet probes of these herbivores have only been documented from permineralizations (i.e. cellular preservation of the internal anatomy), where it is possible to recognize even minor tissue disruptions or wound-callus formation in the immediate vicinity of the probe. In impression and compression fossils, however, such records of tissue disruptions or callus formation in response to attacks are impossible to document based on the nature of the preservation. Since the impression/compression fossil record of Carboniferous seed ferns does not provide reliable data about the damage caused by herbivores, there is currently no basis for estimating the effectiveness of the defence systems used by these plants to ward off plant-feeders. Moreover, in research on extant plant–herbivore interactions, considerable attention is directed at the responses of herbivores to the presence of physical or chemical defence systems in the plants (e.g. Strong *et al.*, 1984; Hulley, 1988; Karban, 1992, and literature cited therein). Unfortunately, in ancient ecosystems, this highly significant aspect in the 'arms race' between plants and herbivores must remain unexplored because it is neither possible to document from the fossil record the extent to which fossil plants with certain defence mechanisms were avoided by herbivores, nor to explore whether herbivores may have been capable of overcoming the presence of the defences (e.g. by developing immunity to toxic repellents or changes in behaviour).

Previous studies on herbivory in early ecosystems have focused on either the herbivores or the damage they caused, or, in a few instances, indirect interactions based on plant symptoms. Little attention, however, has been directed at the defences used by ancient plants to discourage plant-feeding animals. This is especially intriguing since the mechanisms effective in herbivory abatement are highly diverse in plants today. The touch-sensitive glandular trichomes of *Blanzyopteris praedentata* represent the only functional example of a specialized mode of defence in response to herbivory based on fossil plant material. They demonstrate that a Late Carboniferous seed fern discouraged plant-feeding arthropods in a way similar to that found today in some modern flowering plants. Touch-sensitive glandular trichomes represent a defence mechanism that evolved at least twice in plant evolution. This discovery is noteworthy because it provides an exciting opportunity to unravel details of a snap shot during the ancient 'arms race' between plants and certain animals using direct comparisons with plant–animal interactions that exist in ecosystems today. The relationship between specialized trichomes on the seed fern *Blanzyopteris praedentata* and elements of the herbivore fauna offers an insight in the complexity of interrelationships that existed in early ecosystems. Complex trichomes of the type seen in *Blanzyopteris praedentata* have not been found in any other Carboniferous seed fern. We assume that alternative physical or chemical defences must have existed, some of which may have involved other types of trichomes. There are many Carboniferous seed ferns that possessed multicellular non-glandular and or glandular trichomes or emergences (e.g. Oliver and Scott, 1904; Barthel, 1962; Cleal and Shute, 1991; Krings and Kerp, 1998). Although it is likely that at least some of these trichomes were effective in discouraging herbivores, none of them displays a comparatively sophisticated mechanism that can be directly compared with those of modern plants. Today there is considerable attention being directed at the chemical constituents of fossil plants (cf. van Bergen, 1999, and literature cited therein). As techniques are continuously refined, it may become possible to extract information from fossils that can be connected with a system of chemical defences, which constitutes another mechanism of herbivory abatement in the never ending 'arms race' between plants and plant-feeding arthropods.

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