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ABSTRACT

Presence of furanocoumarins and other coumarins on the surface of plant cells indicates their important ecological role both in plant protection and in communication with its environment. Their location on the surface may render the plant unpalatable to herbivores, provides defence against insects attack, controls oviposition and protects from bacteria and fungal spores. Removal from the surface may inhibit the germination of other species. When present on the embryo surface, coumarins may cause autoinhibition and their leaching in water during the spring permits the seeds to germinate. Environmental stresses such as high altitude, extreme temperatures, changes in the UV level and atmospheric pollution influence the production of coumarins and their extrusion to the surface.

Key words: Allelochemicals, coumarins, furanocoumarins, furocoumarins, environment

1. INTRODUCTION

Introduction of the term allelochemical by the American biologists Whittaker and Feeny (35) recognised that many naturally produced substances usually called secondary metabolites have the ability to affect the growth, health, population biology or behaviour of another species. In the intervening period the importance of such substances has increased appreciably. This paper reviews the roles of coumarins as allelochemicals and in the interactions between plants which produce them and the environment.

2. EFFECTS ON HERBIVORES

Realization that coumarins can act as allelochemicals first arose from experiments of Berenbaum (4). They reported an instance of a plant's escape from a lepidopterous larval herbivore, Spodoptera eridania, through its ability to synthesize linear furanocoumarins. This larva does not grow well on Pastinaca sativa, which contains several furanocoumarins, including xanthotoxin and this proved very toxic to it, the toxicity being markedly reduced in the absence of ultraviolet radiation. It thus appeared that the known UV-catalysed reaction of linear furanocoumarins with DNA, blocking the metabolism of the insect, is the cause of the toxicity.

Berenbaum (5) also demonstrated that xanthotoxin was not toxic to Papilio polyxenes, whose host plants from Umbelliferae contain psoralens; in this case the coevolution of the insect with the host plant evidently led to development of tolerance to the psoralen. The biochemical basis for this tolerance has been established as the oxidative furan ring cleavage mediated by the insect's microsomal mixed function oxidases occurs much more rapidly in P. polyxenes than in Spodoptera (9, 10). This is a specific example of a generalized detoxification mechanism utilized by insects to eliminate lipophilic allelochemicals elaborated by plants – their degradation by polysubstrate mono-oxygenases to yield increasingly polar metabolites which are easily excreted (1).

Furanocoumarins appear to be important in communication between these
larvae and their host plants, serving to promote recognition and utilization as food (4, 5). Of interest also is that some relatively advanced tribes of the Umbelliferae elaborate angular furanocoumarins and Berenbaum (6) further showed that these can reduce the growth rate and reproductive ability of _P. polyxenes_, which tolerates and even utilizes the linear isomers. She theorized that development of the biosynthetic pathway to the angular isomers could have been in response to pressures exerted by these specialist larval herbivores.

Extending her studies, Berenbaum (7) surveyed 12 species of Umbelliferae and found that insect herbivores on these plants distribute themselves according to the distribution of furanocoumarins in the host plant. The dominant insects on species lacking these coumarins are generally herbivores, while insects on species having both linear and angular furanocoumarins are dominated by extreme specialists.

Berenbaum and Neal (8) have studied another feature of Oe toxicity to herbivorous larvae by psoralens. Many umbelliferous plants contain another phenylpropanoid, myristicin, which acts synergistically with psoralens. Even 0.1% of this compound in an artificial diet resulted in a fivefold increase in the toxicity of xanthotoxin to _Heliothis zea_, a lepidopterous larva. These workers concluded that plants can enhance the effectiveness of the small amounts of allelochemicals they produce by producing substances that interfere with detoxification, thereby reducing the energy cost of defence and circumventing the development of resistance in herbivores.

Does this phototoxicity observed in insects extend to other herbivores? There is evidence that it does so. Observations have been published that furanocoumarins consumed by grazing mammals such as cattle and sheep, as well as poultry, can photosensitize these animals (20). In humans, however, it appears that dietary consumption of these psoralens in too low, except on rare occasions, for any effect to be observed.

3. COMMUNICATION WITH INSECTS

Stadler and Buser (31) have identified xanthotoxin as an attractant for the carrot fly _Psila rosae_, which, synergistically with other compounds, stimulated the fly to deposit its eggs on the leaf surface. A new dimension was added at this time in that the stimulatory compounds were detectable on the leaf surface, where they would be, of course, ideally situated for communication with the environment.

In contrast, psoralens can exert a negative effect on insects, acting as antifeedants, substances which repel the insect from a potential food source. Several _Spodoptera_ species as well as _Leptinotarsa decemlineata_ and _Mythimna unipunctata_ are affected in this way (21). Such an influence by these compounds obviously can be of great survival value to a plant.
4. DEFENCE AGAINST MICROBIAL ATTACK

Insect herbivores are not, of course, the only organisms which attack plants and a major aspect of the plant's defence is the ability to protect against microbial infection. It is known that linear furanocoumarins and especially psoralen and xanthotoxin, have phototoxicity against fungi (11, 14, 32), bacteria (15, 18, 23, 26), viruses (16, 25) and bacteriophage (33). Because their synthesis is greatly accelerated by the plant in response to wounding, furanocoumarins fall into the category of phytoalexins (11). Thus, Apium graveolens and Pastinaca sativa, for example, respond to infection with microbial pathogens by greater production of psoralens (21). It would appear that the antimicrobial effects of psoralens are not always light-dependent, although photosensitization is undoubtedly the most important aspect of their toxicity. Of incidental interest is that this property of pathogens in eliciting formation of psoralens has proved of great value in studies on the biosynthesis of these coumarins.

5. EFFECTS ON SEED GERMINATION

Another aspect of a plant's communication with its environment relates to other plants. It has been known for many years that furanocoumarins are germination regulators (24, 29, 34). Baskin et al. (2) isolated psoralen from the seed coats of Psoralea subacaulis and showed that it inhibited germination of seeds of four unrelated species as well as that of its own seeds. Friedman et al. (19) demonstrated that xanthotoxin found in Ammi majus had a strong inhibitory effect on the germination of seeds of three other species. No effect was observed on A. majus seeds themselves, a phenomenon which these workers explained by postulating a compartmentation between the inner and outer fruit envelopes, the inner layer preventing access of the phytotoxic xanthotoxin to the embryo. The effect of psoralen on other seeds is an important aspect of allelopathy in that it enables the plant producing psoralens to reduce competition in its immediate environment, while not affecting the germination of its own seeds.

Another aspect of this effect, as in the case of P. subacaulis, is autoinhibition. It has long been known that seeds of some umbellifers are very difficult to germinate. Studies in our laboratory on furanocoumarin concentrations in fruits and seeds of umbelliferous species (41, 42) are consistent with the idea that in the seeds of some members of this family psoralens function as autoinhibitors of germination.

Inhibition of germination by these coumarins can be explained by their ability to inhibit mitosis (18, 30, 45). Zobel and March (47) used auto-fluorescence microscopy to study localization of furanocoumarins in different seed tissues of Rutaceae and fruits of Umbelliferae and Leguminosae. In Psoralea bituminosa they are localized on the fruit surface, outside the embryo cells (48).
Baskin et al. (2) have summarized the ways by which germination inhibitors confer survival value on a species: (a) by preventing premature germination, (b) by extending the period during which germination can occur, (c) by ensuring that an adequate supply of water for seedling establishment is present, and (d) by preventing establishment of other nearby species. Contact of psoralens with embryo cells could result in autoinhibition of germination. We can envisage a scenario in which, during thaws in winter and spring, germination-inhibiting coumarins (and other possible inhibitors) are slowly washed out of the seed, mitosis can then be renewed and germination occurs. This would be especially advantageous to *H. lanatum*, whose habitat is moist locations. At the same time coumarins entering the adjacent soil may prevent germination of seeds of potential competing species.

6. ROLE OF LOCALIZATION IN COMMUNICATION

Localization of coumarins within the plant has an impact on the value of these compounds in communicating with the environment. We have already referred to the role of coumarins as attractants and antifeedants in the relation of plants with insects and indicated that surface deposits are important. Also as mentioned earlier, surface deposits of xanthotoxin on *Daucus carota* were shown to function as attractants for the carrot fly. Ceska et al. (13) later reported having observed microscopic crystals of psoralens on the surface of fresh roots of *Pastinaca sativa*; these were predominantly angelicin, but psoralen, xanthotoxin, and bergapten were also identified. However, little attention was paid to surface coumarins in 1980s due to lack of adequate methodology for removal of material from the plant surface.

We then found that dipping of leaves of *Ruta graveolens* for short period in water near its boiling point removed more furanocoumarins than the conventional techniques of extraction with organic solvents (38). The furanocoumarins are deposited in a thick epicuticular layer of wax (39, 41). This technique showed that in some cases the major part of these coumarins was on the leaf surface: >60% in some cases (42). We later extended this method to numerous other furanocoumarin-bearing species and in all cases found deposits on the surface (51). The amounts varied considerably among species, but in some plants the percentage on the exterior was high even though the total amount was relatively less. The widespread distribution of furanocoumarins even in callus cells (44), suggested their survival value for the plants in this region. This is especially evident when their antimicrobial function is considered, antimicrobial substances on the surface are ideally positioned to act as the first line of defence against airborne pathogens. Clearly it can cause toxicity to man from species containing psoralens, in that mere skin contact with the surface deposits and exposure to UV radiation could induce the photophytodermatitis.

Although the defence role of psoralens would justify the huge energy needed to exude them on the surface. Another advantage in their storage outside the cells is that these compounds are cytotoxic (27, 46), hence, their presence in significant concentrations within the cells would certainly be harmful to the plant. Extrusion to the
surface thus represents a form of compartmentation and provides a means of minimizing autotoxicity, as well as a defence barrier against potentially damaging organisms. Plants, whose excretory functions are very limited, have little choice in ridding themselves of such toxic products of their secondary metabolism, but one another possibility is movement into the intercellular spaces. Although we have obtained indications (unpublished) that this may be an alternative means of compartmentation for psoralens, conclusive evidence is still lacking.

7. EFFECTS OF STRESS ON PRODUCTION OF COUMARINS

7.1 Stress due to Natural Environmental Changes

The effects of normal variations in the environment on the production of coumarins in the growing plant have been explained with studies on Heracleum and Ruta.

7.1.1 Altitude

In the 1970s, French investigators observed no climate–related differences in the metabolic patterns with successive formation of various furanocoumarins between the greenhouse-grown Heracleum plants and those cultivated at an altitude of 700 m (12). This suggests that factors associated with altitude, such as the rarefication of the atmosphere and higher levels of ultraviolet radiation, do not exert influence on the formation of these coumarins. However, in the latter case more controlled experiments have modified this indication, as will be discussed later.

7.1.2 Seasonal variations

In 1988, a season notable in North America for recurring high summer temperatures, we examined concentrations of psoralen, xanthotoxin and bergapten in whole leaves of H. lanatum throughout the vegetative season (40). In this case changes in the production of furanocoumarins due to temperature variations were superimposed on changes occurring during the normal vegetative period. In an individual leaf, the production of these coumarins paralleled the spring growth of the leaf, but then decreased after mid-May in spite of continued leaf growth. But in early July, after five days of above-normal temperatures (30-36°C), there was 3-4 fold increase. Again in the cool weather of late autumn, a considerable increase in the formation of these coumarins was noted and the amounts translocated to the surface were especially higher. These results show the possibility of an effect of temperature stress, although they do not conclusively show effects of high and low temperatures.

7.1.3 Growth in a greenhouse

The plants growing in glasshouse are subjected to conditions which are different from natural conditions. In studies on Ruta chalepensis Zobel (37) found
that transferring the plants from outdoors into the greenhouse led to changed concentrations of furanocoumarins, both in the whole leaf and on the leaf surface. Compared to plants grown all the year in a greenhouse, the concentrations decreased in the first two weeks, afterward they recovered and exceeded those of the plants grown continuously indoors. The changes were also recorded in the proportions of the individual furanocoumarins, with psoralen undergoing a relative decrease. Similar trends were observed in R. graveolens (unpublished). There appears to be two parallel physiological processes influencing concentrations of furanocoumarins in and on the leaf, one genetically dependent and typical of the species which governs concentration levels according to the season and the second an environment-dependent physiological response.

7.1.4 Ultraviolet radiation

Another form of natural stress is due to the ultraviolet radiation from the sun. This has become important owing to fears of ozone depletion in the upper atmosphere. UV radiation reaching the earth's surface through ozone holes for protracted periods at levels exceeding the long-term norm seems certain to have effects on plant growth. Although the nature of the effects to be anticipated is uncertain, there is reason to expect that they could be harmful and result in lower crop yields. However, although UV radiation is stressful but its effects can be beneficial. It is known that UV radiation can raise plants productivity possibly through conversion of its energy to long-wave infrared which could raise growth temperatures (22). In view of this, recent investigations of the influence of UV on the concentrations of furanocoumarins are of interest.

It was observed some years ago (3) that UV irradiation can result in higher concentrations of furanocoumarins in a plant but there was no attempt to differentiate the compartments of the plant surface and its interior. Recently Zangerl and Berenbaum (36) compared the formation of furanocoumarins by Pastinaca sativa in plants screened from UV by a filter with that by plants in full sunlight. In full light the levels of three coumarins were significantly higher than in the absence of UV, while levels of two others were unchanged. Ratios of the coumarins were also affected by absence of UV.

Our recent studies (43, 50) have focussed on the effects of low-intensity, artificial UV radiation as opposed to natural UV of solar origin and have confirmed these findings. Our studies were extended to the effect on compartmentation of these compounds by export to the surface and showed clear effects on furanocoumarin concentrations. Levels of total furanocoumarins in the leaves were increased compared to the lower levels of plants kept in darkness. The effect of scattered radiation falling on leaves was to raise the levels more than the direct irradiation. There was also greater extrusion to the surface, especially in lower, older leaves exposed to the scattered rays.
Presently, we cannot explain this phenomenon. In view of the increased formation of coumarins from unfiltered sunlight, it does not appear likely that our low-intensity source produced radiation levels near it which exceeded the optimum. We may be concerned here with some chemical signal from the most highly irradiated leaves which stimulated synthesis in more remote locations. As even more accelerated extrusion to the surface was observed in the indirectly irradiated lower leaves, we may be seeing here a defence reaction in response to radiation stress. Furanocoumarins absorb UV radiation strongly and when deposited on the surface would provide some degree of protection to the plant i.e. a natural sun screen (50). It has been shown (28) that UV irradiation excites furanocoumarins, and, if we assume that this happens to such compounds exported to the leaf surface, the increased reactivity could render the compounds more active against microbial pathogens, with greater resistance to infection.

7.2 Unnatural Stress: Effects of Air Pollution

Attention may now be turned to the role of coumarins in interaction with an unnatural environment i.e. polluted atmosphere. This subject has not been extensively investigated. The first study appears to be that of Dercks and co-workers (17) who examined the effects of acidic fog and elevated ozone levels, characteristic of urban areas of southern California, USA. They worked on celery, *Apium graveolens*, a major commercial crop in that part of the country, which elaborates psoralen, xanthotoxin, bergapten and isopimpinellin. They simulated acid fog of pH 2 and 3 with a fog-generating apparatus and found stimulation of production, over periods up to five days, by as much as 400-500% in the case of the pH 2 fog. It was a matter of concern that the levels were at least seven times as high as those known to produce contact dermatitis. Over five days ozone at 0.20 ppm for 2 h had no effect, although significant decreases were observed after 24 h.

In our laboratory, we have since investigated the effects of acid and salt sprays on the furanocoumarins of *Ruta graveolens* (49) by a somewhat different experimental approach. In this case spraying was done 10 times at hourly intervals on the first day, and thereafter once a day for one week. The pH 2.5 was comparable to that used by the American workers but in our case, sulphuric acid alone was used, whereas they had used a nitric-sulphuric mixture and our plants were not analysed until 14 days after the first treatment. Over this period, the total furanocoumarin concentrations decreased very markedly in the upper leaves for the three compounds examined: psoralen, xanthotoxin and bergapten, with lesser decreases in the lower leaves. These decreases were in contrast to the very marked increases in concentration observed in celery. We do not know whether the discrepancy is due to a species difference, a difference in the duration of the study, or possibly to the differing acid composition. Sodium chloride spray also led to decreases in furanocoumarin concentrations of the same order as we observed with the acid spray.
In view of the importance of this compartment in the present context, we examined the concentrations on the leaf surface separately. After the acid spray, total furanocoumarins decreased in the upper leaves by a factor of >2 compared to the unsprayed control, but in the lower leaves they increased by a similar factor, resulting in 70% of the total furanocoumarins being on the surface. After the salt spray, a similar decrease was observed in the upper leaves, while concentrations in the lower leaves were little affected. Scanning EM revealed cracks in the epicuticular layer. The fact that more numerous cracks were noted in the upper leaves perhaps accounts for easier penetration of the pollutant into these leaves and thus greater retardation of their physiological processes.

In conclusion, it was found that a genome exists, which controls the synthesis of coumarins in the cell. The extent to which the genes are expressed is greatly influenced by natural and unnatural environmental factors, which interact with the plant. Such environmental influences can exert either positive or negative effects, not only on the formation of coumarins but also on their compartmentation, or in translocation to the surface. There is evidence that some coumarins act as phytoalexins, whose concentrations are elevated in response to wounding and exert an antimicrobial effect on invading pathogens. When extruded to the leaf surface they form the first defensive barrier against such invasions and also form a shield against potentially harmful UV irradiation. The inhibitory effects of some coumarins on seed germination can reduce competition from other species in appropriate instances but in certain cases autoinhibitory influences can also delay germination of the plant's own seeds until growth conditions are favourable. Coumarins are classified among products of secondary metabolism, whose functions have long been a subject of debate. In recent years, however, the survival value of these compounds is becoming more evident. In fact, we now know that, in the interaction of coumarin-producing plants with their environment, coumarins are indeed of primary importance and thus needs increasing attention which is being given to them.

REFERENCES


Coumarins in plant and environment