MONTANA STATE UNIVERSITY

Biology of the eremophilanes produced by Drechslera gigantea
by Gregory James Bunkers

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Pathology
Montana State University
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Abstract:
Drechslera gigantea. the causative agent of zonate eyespot disease on grasses, produces at least twelve bioactive molecules known as eremophilanes. Their structures have been elucidated using conventional spectroscopy and x-ray crystallography. A study to examine the biological aspects of these eremophilanes was undertaken.

A procedure, using high performance liquid chromatography to quantify eremophilane levels in culture filtrates of D. gigantea was developed. This procedure was used to study eremophilane production by D. gigantea under different cultural conditions. Leaf material from quackgrass (Agropyron repens), a host of the fungus, stimulated toxin production. Amendments such as L-leucine or the sterol biosynthesis inhibitor chloro-choline chloride (CCC) also exhibited stimulatory activity.

The structure-activity relationships of the eremophilanes were investigated using three different bioassays. No distinct functional groups or structural characteristic could be correlated to activity. However, in all three bioassays, the eremophilanes with the higher oxidation states were generally less active.

To determine the mode of action, the effects of eremophilanes on the physiology of the plant were studied. Eremophilane bioactivities mimic the activity of known phytohormones. Comparative studies indicated that these activities seem not to be associated with induction of known phytohormones but are inherent properties of the eremophilane molecules. The eremophilanes were shown to inhibit protein synthesis both in vitro and in vivo. The proposed mode of action of the eremophilanes is inhibition of protein synthesis.

Finally, the fate of the eremophilanes in planta was investigated. [14C]-petasol was applied to detached oat leaves (Avena sativa cv. Park) and the radiolabel was traced. The [14C]-petasol was converted to a compound which exhibited different chromatographic properties than petasol and was not bioactive. NMR analysis indicated that the petasol moiety was present in the conversion product. Hydrolysis of the conversion product led to the recovery of petasol. Amino acid analysis indicated that amino acids were also present in the hydrolysate. Results indicate that the plant is capable of modifying this eremophilane to form a petasol-amino acid conjugate.

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ABSTRACT

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CHAPTER I

INTRODUCTION

Higher plants and their pathogenic fungi have evolved together throughout millennia, with their dynamic biochemical interchanges constantly adjusting. Included in the biochemical interchange are fungal-produced phytotoxins. The concept that plant pathogens produce pathogenic toxins originated about a century ago (62). Gaumann (29) concluded that microorganisms responsible for disease act by virtue of the toxins they produce, however, that phytopathogens induce disease through toxigenic action has not been rigorously proved. Arguments for their participation in pathogenesis center on two main points: 1) some toxins are host specific and loss of ability to produce the toxin in culture is accompanied by loss of pathogenicity; 2) the symptomatic effects of the toxin are so distinctly similar to all or part of the disease syndrome that there can be little doubt that the toxin is involved. The other side of the argument has been stated quite succinctly by Day (16) who commented that pathogenicity based on toxin production may not be a major mechanism, but rather an evolutionary fluke.

An understanding of the relevance of toxins in disease expression by plant pathogenic microorganisms has been slow

to develop. This has largely been due to the lack of understanding of the chemistry involved, or of the necessity for chemical purity. Ultimately the goal should be to define plant pathogenesis at the molecular level; studies using chemically characterized toxins provide a powerful tool and useful starting point for reaching this objective.

Pathogen produced toxins have also found application in many other areas of plant pathology including use as models for studies of disease physiology (58), screening for resistance among populations of plants (10), and perhaps the greatest use is as experimental tools in selecting for disease-resistant cells or tissue in vitro (30).

The practical significance of pathologically important toxins is being further recognized as more applications become apparent. The chemical specificity of some toxins in particular make them extremely good metabolic probes for investigating cellular and enzymatic functions. Fusicoccin, a toxin produced by Fusicoccum amygdali, is a prime example of a toxin that is proving exceedingly useful for studies on plant metabolism. Basically, it stimulates membrane bound ATPases which leads to a proton electrochemical gradient established across the membrane (42). This primary event leads to a number of effects on different physiological processes including proton extrusion, cell enlargement, increased respiration, dark CO_2 fixation and ABA antagonism (42).

Some toxins have been proposed for use in taxonomy. Tentoxin, produced by Alternaria tenuis, has been used in taxonomic applications involving higher plants, utilizing its property to inhibit the chloroplast coupling factor (CF_1) only from selective plants (9). This selective inhibition was used to determine species ancestry in cases where the putative parents vary in their reaction to tentoxin.

High molecular weight polysaccharides produced by Xanthomonas and Pseudomonas spp., which act as wilt inducing toxins, have found commercial application. In particular "xanthan" gum, produced by X. campestris pv. campestris, is used in a wide variety of commercial applications (23). Pseudomonas spp., as well as several fungi, produce similar exopolysaccharides which have been characterized as proteinaceous and polymerized DNA heteropolysaccharides (73). It is likely that because of this rich diversity, more commercial use will be made of these substances and more organisms will be surveyed for their production and properties.

There is much work being done to develop weed pathogens as weed control agents. Ideally, the pathogen will establish itself and reach epiphytotic levels thus reducing the weed population to subeconomic levels. Skeleton weed (Chondrilla juncea) has been successfully controlled in Australia by the introduction of the rust fungus Puccinia

chondrillina from the region where the weed originated (6). Attempts to duplicate this success are now being made throughout the world (6, and refs. therein). Mycoherbicides, plant pathogenic fungi developed to control weeds, are available commercially in the United States. DeVine, a formulation of Phytophthora palmivora used for control of strangler vine (Morrenia odorata), and Collego, a formulation of Colletotrichum gloeosporiodes f. sp. aeschynomene used for control of northern jointvetch (Aeschynomene virginica), are being used for weed control in the southeastern United States (66).

Use of live pathogens as biocontrol agents is not without risk. The pathogen may actually prove more virulent on important crops in the surrounding area than to the desired target weed, risking dire results. Containment of the pathogen to the targeted area would be difficult because of the somewhat unpredictable manner of such dispersal mechanisms as wind, water, and animal vectors (3). Although the "escape" of these control agents is a potential, the more realistic concern is actually the inability of a chosen pathogen to propagate disease in a target area. Many obstacles must be overcome including inoculation, genetic diversity of the host, and unpredictable environmental factors such as temperature and humidity. These difficulties in using live phytopathogens as control agents have led to increased interest in phytotoxins produced by

weed pathogens.

Potential applications of toxins produced by weed pathogens have only recently been realized. If weed pathogens, which produce host-specific toxins, can be identified it could lead to weed control with natural or synthetic compounds having a narrow spectrum of activity and minimal impact on the environment. A desire for selective, environmentally low impact herbicides has generated increased research activity in phytotoxins produced by weed pathogens as potential chemicals for the herbicide industry (22). At the very least, natural compounds may suggest structural types and arrangements of functional groups that could serve as starting points for the development of improved herbicides.

Our laboratory has focused on weed pathogens with hope of finding both novel and more selective chemical control agents. This led to the study of Drechslera gigantea, the causal agent of zonate eyespot disease on numerous grasses (21). This leaf disease is a serious problem in many commonly cultivated turfgrasses and also occurs in weeds such as crabgrass (Digitaria spp.), quackgrass (Agropyron repens), and bermudagrass (Cynodon dactylon). Drechsler (21) pointed out that during the summer of 1922 it appeared in the vicinity of Washington, D.C., as probably the single most destructive parasitic fungus affecting the Gramineae family. He also suggested that in nature there were no

physiological varieties or races paralleling generic divisions in the Gramineae. Dale (14), however, did indicate that several lines of bermudagrass have been introduced that show some degree of resistance to the disease.

Symptoms of zonate eyespot disease first appear as brown flecks, often not exceeding 0.05 mm in width and 0.2 mm in length (13). These flecks eventually enlarge and fade to a greyish oval lesion surrounded by reddish brown or dark green borders thus suggesting the name "eyespot". When these lesions become numerous and coalesce causing the leaf to dry somewhat, conidiophores appear around the periphery of the lesions as well as from the bleached area in the center of the lesion. In nature, Drechsler (21) found a peculiar type of secondary development which led to production of copious numbers of spores. During times of heavy dews or prolonged periods of water persisting on infected foliage many of the lesions could be found to be surrounded by a water soaked zone supporting growth of superficial mycelia. With the onset of drier conditions growth of the superficial mycelium ceases and abundant sporulation occurs. Rather meager sporulation in culture led Drechsler to speculate on the importance of this secondary development on sporulation.

When grown in liquid culture, D. gigantea produces several bioactive terpenoids (28,37). Terpenes constitute