Development of an Endemic Fungal Pathogen as a Mycoherbicide for Biocontrol of Northern Jointvetch in Rice[1]

by
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Northern jointvetch (*Aeschynomene virginica* (L.) B.S.P.) is a troublesome leguminous weed in eastern Arkansas in rice fields, canals and waste areas; on field levees and ditch banks; and to a lesser extent in soybean fields (Smith & Shaw, 1966). This weed reduces yield and quality of rice. Populations as low as 2.7 plants per square meter reduced yields significantly when competition lasted all season (Smith, 1968. Competition was most evident when the weed began to shade the rice at 8 to 10 weeks after emergence. The grade of rice grain is reduced if it contains northern jointvetch seeds. Seeds are very difficult to remove from rough rice in the milling process and are intolerable in milled rice (Smith & Shaw, 1966).

Origin of the weed in America is uncertain but it probably is native. It is widely distributed in the rice-growing region of the state along with Indian jointvetch (*A. indica L.*), but the latter predominates in uncultivated areas, canals and ditches, while the former is more prevalent in rice fields. Northern jointvetch was first described by Linneaus as *Hedysarum virginicum* in 1753 from collections made in Virginia. In 1913, it was listed by Britton and Brown as being distributed in "Southeastern Pennsylvania, Southwestern New Jersey to Florida west to Louisiana, Mexico and Jamaica" (Britton & Brown, 1913). Rudd (1955) implied that many of the collections from the south and western U.S. are, in fact, *A. indica* and that the range of *A. virginica* at that time was eastern U.S. Northern jointvetch was first described as a weed in Arkansas rice fields in 1920 (Chambliss, 1920), and currently it infests approximately 125,000 acres of rice in Arkansas, northern Louisiana and Mississippi.

Northern jointvetch plants are erect annuals up to 2 meters tall and have hisped branched stems (Smith & Shaw, 1966). Leaves are pinnately compound on a sparsely hispid rachis. Membranous stipules, 10 mm long, are acute at apex and base. Pods are flattened and segmented into 5 to 10 nearly square indehiscent sections (joints) containing seeds that persist in harvested grain and infest soils for 20 years or more. Seeds do not germinate under water, but germinate while the field is drained.

The weed is presently controlled in rice by propanil and phenoxy herbicides. If not applied at the optimal time, these herbicides may injure rice or fail to control the weed (Smith & Shaw, 1966). Phenoxy herbicide treatments may increase the susceptibility of rice to brown leaf spot (*Helminthosporium oryzae* B. de Haan) (Smith & Templeton, 1968). Also, aerial application of phenoxy herbicides frequently results in spray drift from the target rice fields that may injure nearby susceptible crops, especially cotton and soybeans (Smith & Shaw, 1966).

Northern jointvetch anthracnose (*Colletotrichum gloeosporiodes* f. sp. *aeschynomene*) was first noted in 1969 at the Univeristy of Arkansas Rice Branch Experiment Station at Stuttgart, Arkansas (Daniel et al., 1973) (Daniel et al., 1974). The disease virtually eradicated these weeds. The natural distribution of the disease on northern jointvetch was determined by surveying rice fields, canals and drainage ditches in rice-growing areas of Arkansas during 1970 and 1971 about the time weeds matured (Smith et al., 1973). Diseased weed plants were found wherever the weed occurred throughout the rice-growing region of Arkansas, but the infection did not occur at a sufficiently high level to kill individual weed plants.

The organism produces typical anthracnose lesions with abundant sporulation from acervuli

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throughout the lesions (Daniel et al., 1973). On mature plants, these lesions range from 0.9 to 3.5 cm long, sometimes coalesce, and frequently develop at the leaf axil. They may be scattered on individual plants in the colony. Natural infections appear to be of minor consequence to the weed because the disease does not reduce vigor of seed production appreciably.

The disease cycle is probably identical to that of many seedborne anthracnose diseases of economic crops and native plants. The organism overwinters in seeds and infests host plant debris as spores or mycelia. Primary inoculum from these sources infects cotyledons and stems of young seedlings in the spring. Although acervuli are produced abundantly in lesions on young plants, release of conidia is suppressed by the host epidermis that usually persists over the acervuli in young tissue. Futhermore, sticky conidia are not readily wind blown, but require splashing rain or insects to disseminate them. The disease peak coincides with host plant maturity and is finalized by seed infection or infestation to complete the life cycle. The balance between the pathogen and its host unfortunately favors the weed or host plant. In nature, the disease fails to give enough control to prevent economic threshold levels of the weed. Low overwintering levels of the pathogen and poor dissemination of spores are the major factors that prevent it from reducing weed populations to suitable levels in the intensively cultivated rice crop.

The fungus was easily isolated from infected northern jointvetch plant tissue (Daniel et al., 1973). It grew rapidly and sporulated abundantly on potato-dextrose agar, lima bean agar, and many other commercially available nutrient media. Lima bean agar was the solid medium selected for routine identification and growth. The optimum temperature for linear growth on lima bean agar ranged from 28 to 30C, with no measurable growth at either 16 or 40C. Stock cultures of the fungus were maintained on sterile soil in tubes at 10C.

Liquid media were preferable for mass producing spores (3). Continuous aeration by vigorous shaking was necessary for maximum yields of spores in liquid media. Highest spore yields were obtained when the fungus was grown for 5 days in modified Richard's solution, placed in a 250-ml flask on a rotary shaker (110 rpm) at 30C. Scale-up of this procedure to commercial tank fermentors has been accomplished by the Upjohn Company[3].

Virulence and specificity experiments were conducted in greenhouse tests with seedling plants of numerous species (Daniel et al., 1973). Seedlings were inoculated by spraying until runoff with water-spore suspensions containing 2 x 10^6 spores per ml. Inoculated plants were then held in a moist chamber overnight before being returned to the greenhouse. About 5 days were required between inoculation and development of disease symptoms, including acervulus production in favorable greenhouse environments. Northern jointvetch plants ranging from 5 to 30 cm tall were infected and killed by the organism. Although the fungus infected and stunted Indian jointvetch plants, it did not kill them. Spore inoculations produced no infection on 165 crop and native plant species.

The efficacy of the fungus for controlling northern jointvetch has been tested in replicated field experiments from 1970 through 1976 and in commercial rice fields from 1973 through 1976. In field plots, control was achieved by inoculum spore concentrations of 2, 4, or 6 million spores per ml when sprayed at dusk at 374 L/ha (Daniel et al., 1973). Control was most rapid when weeds were young, but control averaged 99 percent when weeds were as tall as 66 cm. In 1972, northern jointvetch plants were controlled in a 1.2 ha field of sed rice by aerial spraying with a concentration of 2 million conidia per ml in 94 L/ha of water. Weeds were 30 to 45 cm tall and disease symptoms developed after 7 days. In the treated field, the disease killed 99 percent (4,430 of 4,490 were killed) of the weed plants 46 days after treatment. The infection progressed with time; 47 percent of the plants were dead 16 days after treatment and 84 percent were dead 33 days after treatment. Plants not killed were severely diseased and stunted. They did not compete with the rice and produced few, if any, seeds. In an untreated area about 250 m from the treated field, northern jointvetch plants exhibited no symptoms of disease.

Commercial fields were treated in 1973 and 1974 under an experimental permit from the Arkansas State Plant Board. Control ranged from 95 to 100 percent in 4 fields that totaled 32 hectares (Templeton et al., 1974). An experimental use permit and temporary exemption from requirement of a tolerance was obtained from the U.S. Environ-

[3] Rickard, S. F. 1975. Personal Communication. Upjohn Company. Kalamazoo, MI.

mental Protection Agency for 1975 and 1976 field treatments (Templeton, 1975). Spores for these large-scale field test have been produced in standard commercial fermentors by the Upjohn Company.

Efficacy of the fungus was again demonstrated in 1975 with 17 fields of rice totaling 240 hectares. Control ranged from 95 to 100 percent with fresh (not dried) spores. Control with dried spores has been comparable when applied on the basis of equivalent viability. In the 1976 testing program emphasis has been placed on the use of spores in a dried formulated product.

No biological or technological reasons have been encountered or can be foreseen that would preclude commercialization of this fungus as a mycoherbicide.

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