SOLUTIONS TO SOME PROBLEMS IN
ASPERGILLUS TAXONOMY USING THE
SCANNING ELECTRON MICROSCOPE

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SUMMARY

Data obtained from a scanning electron microscope (SEM)
study of intraspecific and interspecific variation in
Aspergilli associated with post-harvest storage are used to
resolve some difficulties in species identification and deli-
mitation. Solutions are provided to problems of identifying
isolates of Aspergillus flavus, A. parasiticus and A. oryzae,
while the status of A. sojae is resolved. The morphological
features of both the conidia and ascospores of A. glaucus are
evaluated as marker characters and are shown to be equally
useful either complementing one another or acting indepen-
dently. The long-standing, intricate puzzle surrounding A.
melleus, A. quercinus and A. petrakii is explained satisfac-
tory. Finally, mycologists employing SEM techniques are warned
of the wide variability that arises from the ontogenetic
development of conidia, and cautioned to ensure that micro-
graphs of mature conidia are obtained.

INTRODUCTION

Problems relating to species delimitation and determina-
tion in the Aspergillii arise from a variety of factors.
Foremost are (1) the almost unwavering confidence with which
mycologists rely upon subjective decisions to differentiate
colony characteristics, and (2) the limited ability of the
light microscope to adequately resolve micro-morphological
features. These difficulties are compounded by the consid-
erable inherent, as well as non-inheritable variability exhi-
bited by isolates belonging to the same taxon.

In a recently completed revision (Kozakiewicz, in press) of
Aspergillus species associated with post harvest storage, the
use of the scanning electron microscope (SEM) has eliminated
the problem of inadequate microscope resolution. A feature of
this work has been to use the genetic integrity exhibited by
the morphology of the conidium to assess the importance and
implications of the considerable variability associated with commonly emphasised characters such as colony appearance. As a result, new data have become available which enables certain mistakes and contentious points in Aspergillus taxonomy to be unequivocably resolved.

METHODS

Conidial samples were prepared by passing an aluminium specimen stub (Agar Aids) across the surface of a mature Petri dish plate colony. The stub was then gold coated (Emscope Sputter coater), examined in a Cambridge Scientific S250 scanning electron microscope at an accelerating voltage of 20kv and photographed using Ilford FP4 35 mm film.

RESULTS

Aspergillus flavus group

An important group within the Aspergilli is the A. flavus group, which includes A. parasiticus, A. sojae, A. flavus and A. oryzae. The identity and separation of these has concerned mycologists for many decades. It has been stated that there are no sharp line of demarcation between, on the one hand A. flavus and A. parasiticus and on the other A. flavus and A. oryzae (Thorn and Raper, 1945; Raper and Fennell, 1965). Koza-kiewicz (1982) has considered the difficulties of differentiating A. flavus and A. parasiticus, but it is pertinent to reiterate some of those findings here. Differentiation of these two species depends on colour, stipe length, presence or absence of metulae and conidial ornamentation. SEM studies of conidia taken from ex-type cultures and various strains labelled as one of these two species revealed two distinct spore forms. Conidia taken from a particular isolate always consisted of one of these two forms, never a mixture of both. One form occurred in the ex-type isolate of A. parasiticus, and a neotype for A. flavus based on the second conidium form was selected (Fig.1). Use of this new diagnostic character revealed that the ex-type cultures of A. parasiticus, and a neotype for A. flavus based on the second conidium form was selected (Fig.1). Use of this new diagnostic character revealed that the ex-type cultures of A. parasiticus and A. oryzae (Thom and Raper, 1945; Raper and Fennell, 1965). Koza-kiewicz (1982) has considered the difficulties of differentiating A. flavus and A. parasiticus, but it is pertinent to reiterate some of those findings here. Differentiation of these two species depends on colour, stipe length, presence or absence of metulae and conidial ornamentation. SEM studies of conidia taken from ex-type cultures and various strains label-

Problems assigning isolates to either A. flavus or A. oryzae are equally acute because it is considered that these species are 'bridged almost completely by a series of intermediate forms' (Raper and Fennell, 1965: 70).

I have examined isolates which have been crucial in the development of the taxonomy of A. oryzae, including one (= NRRL 447) believed to be derived from that used in the original description (Cohn, 1884) and ones originating from the soy sauce industry; all possess conidia ornamented as in Fig. 1. With the exception of one isolate (WB 453) originating from Brazil nuts (Thom and Church, 1926: 201), all others I have examined from sources outside these parameters proved to be misidentifications of A. parasiticus or A. flavus. In general, colonies of A. oryzae isolates can be recognized by