Application of image analysis for variety testing of mushroom

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Summary

The application of image analysis for variety testing of mushrooms to grant plant breeders’ rights was investigated. The measurements of length, width and a range of several other more or less complex shape descriptors determined by means of image analysis were statistically analyzed. A total of 460 observations were used in this experiment. Distinction between 80% of the cultivars in this experiment could be made, at a 1% significance level, using only 4 characters of 44 characters tested. Image analysis methods are a faster and more accurate manner to distinguish cultivars than the standard visual and manual ones presently in use.

Introduction

The application of image analysis in agriculture and plant science is mentioned by several authors (Price & Osborne, 1990; Heijden, 1990; Heijden et al., 1989; Draper & Keefe, 1989; Kranzler, 1985). The technique can replace subjective and time-consuming manual assessments by screening several morphological characters at one time in a quantitative, objective and non-destructive way. Image analysis lends itself for computerization and thus enables the processing of large amounts of material for examination in a relatively short time (Baum & Bailey, 1987). Furthermore, the obtained characters are morphological in nature as are the majority of characters in use for variety testing and registration. Therefore it is more appealing to the variety expert than molecular techniques are, such as electrophoresis, which often have no morphological interpretation. The acceptance of newly introduced characters based on their suitability for machine measurement by national and international guidelines should not prove as much a problem as is the case with biochemical characters (Draper & Keefe, 1989).

For granting plant breeders’ rights (PBR) and the registration of new cultivars of mushroom, Agaricus bisporus (Lange) Imbach, a cultivar-model of mushroom had to be developed. In 1987, 1988 and 1990, at RIVRO, an attempt was made to distinguish several mushroom cultivars by morphological and physiological characters. In this experiment hybrid varieties from white and off-white varieties were grown. The closely related hybrid varieties ‘Euro-Semy 170’, ‘Le Lion X13’ and ‘Hor-witu’ were strikingly similar. These three are different from the other varieties which were only gradually distinct from each other. These small differences are explained by the limited number of morphological characters of Agaricus bisporus and the very small genetic variation in this cultivated mushroom (Van der Neut, 1991). The manually

1 The use of trade names in this publication does not imply endorsement of the products named nor criticism of similar products not named.
assessed morphological data from these tests are insufficient to make a distinction between all cultivars. In this study, image analysis in conjunction with pattern recognition was used to improve the number of morphological characters for distinction between the closely related cultivars of this species. The term image analysis is usually reserved for computerized procedures and may be defined as the capture of an image, followed by the quantification and classification of components within the image. Pattern recognition techniques can be used to construct decision rules which enable units to be identified on the basis of their measurement patterns. Pattern recognition techniques can be employed to cluster together units, the cultivars, having similar measurement patterns (Haralick & Shapiro, 1989).

Material and methods

In 1990 an experiment was set up in a so called mushroom-cell. Compost and casing soil were standard and obtained from the Co-operative Dutch Mushroom Growers Society (CNC). The temperature and humidity in the cell were according the standard used at the Experimental Station for Mushroomculture Horst (Van der Neut, 1991). During the development of the sporophore the air and substrate temperature were kept at 19°C. Spawn of commercially available cultivars was used in this test. The application of image analysis included the cultivars:

A) 'Horwitu'
B) 'Somycel 205'
C) 'Claron AX31'
D) 'Claron A5.1'
E) 'Le Lion X13'
F) 'Le Lion X20'
G) 'Royal 26A'
H) 'Le Champignon 200'
I) 'Euro-Semy 170'

From these nine cultivars, eight open sporophores from the third flush were examined, in 2 to 5 replications. With a video camera an image of a transverse section of each sporophore was recorded, yielding 230 observations. The stem was mechanically removed from the sporophore and these 230 stem observations were recorded separately. The image analysis unit and its associated software consists of the following components: CCD camera SONY XC 77 CE, using a 35 mm NIKON lens, SUN 4/110 SPARC computer and a Data Translation framegrabber DT 1451. The image recording and processing is controlled by the software package TCL-Image version 4.5. The software package Acuity™ is used for the measurements and is an integral part of TCL-Image. Statistical analysis in this study was done on an VAX-3600 mainframe computer running GENSTAT 5.1 statistical software.

Several recording techniques were tested in order to increase the number of morphological characters that can be measured on a sporophore. The aim was to segment the image into background and three objects: one cap (pileus) and two gills (lamellae) with a minimum of grey level preprocessing such as shadow removal. Sporophores were therefore recorded at different illumination and with different filters in front of the lens. The grey level histograms of the thus obtained images were compared. Maximum resolution was obtained with a black, non-reflecting background, frontlight with a colour temperature of 5000K and a red-filter – 5 x in front of the lens (Figs. 1, 2). Care was taken to minimize ambient light and shading effects. The signal from the camera was digitized by an analogue-to-digital converter into an array of picture elements or pixels. This was done in the unofficial standard array of 512 x 512 pixels by the framegrabber. Each pixel is assigned a grey level which represents the brightness or intensity of the image at that point in the array, usually 256 levels where nought is black and 255 is white. To obtain square pixels from the 4 : 3 video standard a multiplication in the y-direction with 4/3 was performed, necessary to make the recording isotropic. A binary image was derived from the grey level image where pixels are either black or white by a process known as thresholding or segmentation. In this way a distinction was made between the objects (pixel value 1) and the background (pixel value 0). Two differ-