# IDENTIFICATION OF MUSHROOM CULTIVARS USING IMAGE ANALYSIS

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## ABSTRACT

The application of image analysis for variety testing of mushrooms [*Agaricus bisporus* (Lange) Imbach] to grant plant breeders' rights was investigated. Images of 230 mushrooms were recorded under controlled lighting conditions. Image analysis techniques were used to increase the number of morphological characters that can be measured. The measurements of length, width, and a range of more or less complex shape descriptors, determined by means of image analysis, were statistically analyzed. Image analysis exhibited a significant improvement over previous methods which used only manual assessments.

**KEYWORDS.** Image analysis, Variety research, Mushrooms.

## INTRODUCTION

Since Dutch plant breeders' rights (PBR) are open to cultivated mushrooms [*Agaricus bisporus* (Lange) Imbach], 11 applications have been submitted. In order to grant PBR to a new application it has to be distinct, uniform, and stable (Fikkert, 1985). This is for the greater part determined on morphological characters (UPOV, 1979).

In plant sciences, and variety research in particular, many interesting morphological characters are not determined quantitatively because their observation is too laborious. Some of these characters are therefore only classified visually or even not recorded at all. Visual classification entails a certain degree of subjectivity and statistical analysis techniques are less powerful for these kinds of data. With image analysis these characters can be assessed in a quantitative and objective way. Image analysis lends itself to automation and thus enables the processing of large amounts of material for examination in a relatively short time (Baum and Bailey, 1987).

The application of image analysis in agriculture and plant science has grown considerably in recent years (Zayas et al., 1990; Price and Osborne, 1990; Heijden, 1990; Draper and Keefe, 1989; Kranzler, 1985). The objective of this article is to demonstrate the usefulness of image analysis in variety testing of mushrooms.

From earlier experiments, with manually assessed data, it was concluded that it was difficult to collect reliable

morphological data of mushrooms in the button stage. In this stage, the sporophore is growing rapidly by stem elongation and cap expansion (Neut, 1991). For that reason, only relatively mature, flat, open sporophores were observed in this experiment.

## MATERIAL AND METHODS

In order to develop a possible PBR test by morphological characters, an experiment was set up in 1990 in a "mushroom-cell". In this cell, with beds of sterile compost, the temperature, humidity, and light are completely controlled. Growing conditions were established according to the standard used at the Experimental Station for Mushroomculture Horst (Neut, 1991). Spawn of commercially available cultivars was used in this test. For the application of image analysis, nine cultivars were selected in order to obtain eight open sporophores in two to five replications for each cultivar. A slice, 3-4 mm thick, was taken from the center of each sporophore. An image of each slice was recorded with a videocamera, yielding 230 observations (fig. 1). The stem was cut 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 (Charge Coupled Device) camera SONY XC 77 CE, using a 35 mm NIKON lens (distance 109.8 cm, f/5.6 lens aperture), SUN 4/110 SPARC computer and the Data Translation frame-grabber DT 1451 with 256 grey levels where 0 is black and 255 is white (fig. 2). The image recording and processing is controlled by the software package TCL-Image. The software package Acuity is used for the measurements and is an integral part of TCL-Image.

Several recording techniques were tested in order to increase the number of morphological characters that can be measured. The aim was to segment the image into background and three objects [one cap (pileus) and two gills (lamellas)] with a minimum of grey level preprocessing such as shadow removal. Therefore, care was taken to minimize ambient light and shading effects and sporophores were recorded with different illumination conditions and with different filters in front of the lens.

The video signal from the camera was digitized by the frame-grabber into an array of picture elements or pixels. 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 0 is black and 255 is white. The grey level histograms of the images were compared for the various lighting conditions. Maximum resolution was obtained with a black, non-reflecting background, front-light with a color temperature of 5000K and a red-filter_5x in front of the lens. In figure 3a the grey level histogram of a
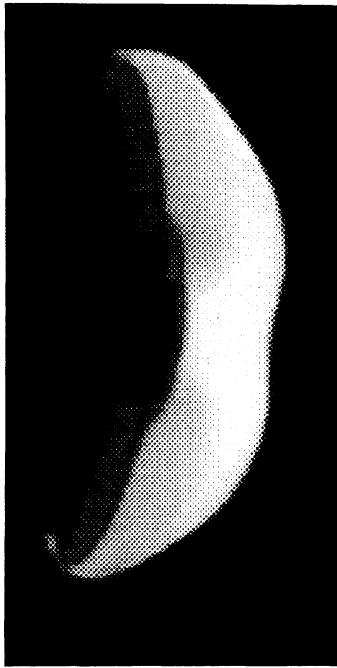
Figure 1–Grey level image of sporophore recorded with a red-filter_5x.



Figure 2–Diagram of the vision system.

of the foreground and the background pixels are calculated separately and a new threshold value is taken midway between these two average values. This process is repeated until the threshold value does not change anymore.

The two bitplanes that keep the results from both the thresholding operations are compared, pixel by pixel. If they match a zero, otherwise a one, was written at the corresponding position in a third bitplane. This so-called XOR operation followed by a closing resulted in the cap and gills as separated objects. The result of these operations is shown in figure 5c.

sporophore recorded without filter is shown and the contrast-stretched, grey level histogram of the same image is shown in figure 3b. Contrast stretching is an operator that, based upon the histogram of the pixels of the image, results in an enhancement of the visibility of an image's detail (Haralick and Shapiro, 1991). Furthermore, contrast stretching compensates for differences in the dynamic range of the various lighting conditions and makes visual comparison easier. The comparison of the two contrast-stretched images (figs. 3b and 4b) shows the effect of the red-filter_5x. The peak at grey value 30 (fig. 3b) has shifted to grey value 60 (fig. 4b). The thus obtained space between the peaks made it possible to separate the gills from the cap by means of thresholding.

A binary image, where pixels are either black or white, can be derived from the grey level image by a process known as thresholding or segmentation. Thresholding produces a binary one on the output image whenever a pixel value on the input image is above a specified minimum threshold level. A binary zero is produced otherwise. In this way a distinction was made between the objects (pixel value 1) and the background (pixel value 0). Two different threshold levels were used.

With a first fixed threshold cap and gills were separated from the background (fig. 5a). A closing operation was performed to smooth the contours, fuse narrow breaks, eliminate small holes, and fill gaps in the contour. A closing is an operation which consists of a dilation of the border of the binary object with a certain number of cycles, followed by an erosion of that border with the same number of cycles.

The separation of the cap from the background and gills was established after a second threshold using the isodata algorithm (fig. 5b). The isodata algorithm is an iterative method. The histogram of a grey level image is split into two parts, the foreground pixels and the background pixels, assuming an initial threshold value. Then the average values
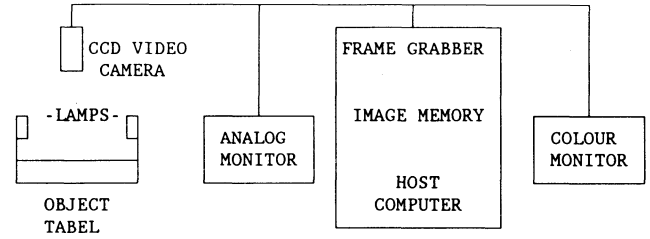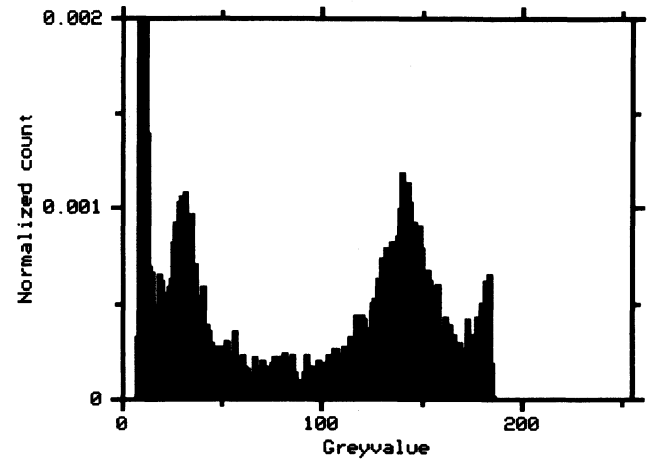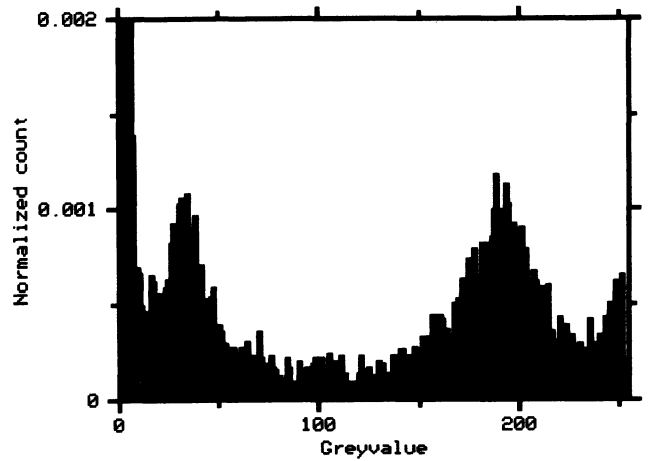


(a)



(b)

Figure 3–(a) Grey level histogram of sporophore recorded without filter and (b) grey level histogram after contrast stretching.
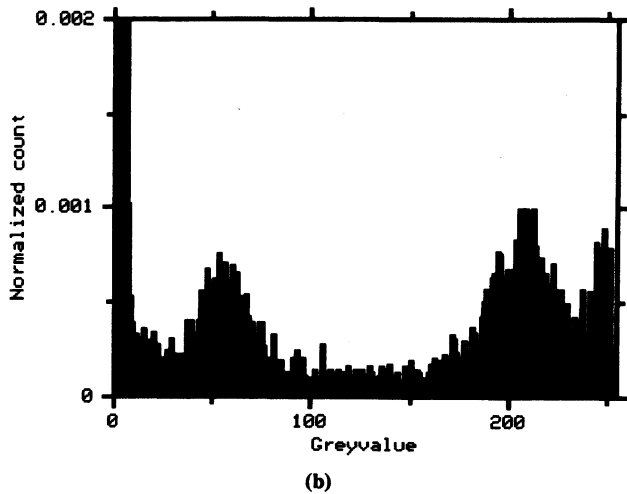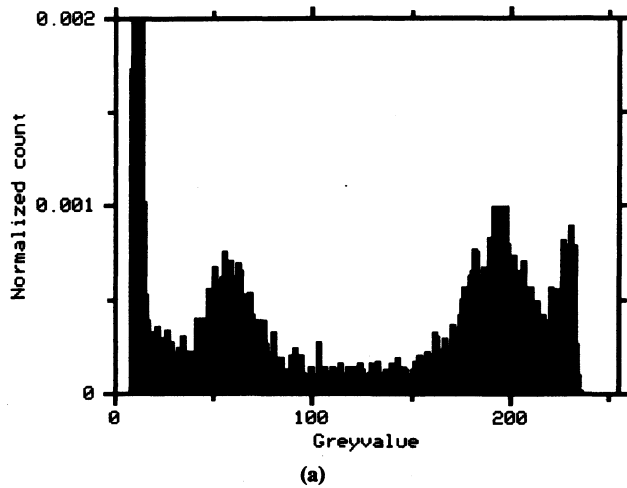
(a)



Figure 4–(a) Grey level histogram of the same sporophore with a red-filter_5x and (b) the grey level histogram with red-filter_5x and after contrast stretching.

To remove small objects, an erosion was performed, followed by a propagation in order to bring back the original object sizes of the gills. A propagation lets object kernels grow, by repeated expansion, with the condition that the resulting pixels stay within the borders of a mask object. The image before the erosion was used as the mask. The result is that small objects have disappeared without altering the scope of the main objects. Closing of this object resulted in the smoothed images of the gills on both sides of the transverse cap (fig. 5d).

It was thus possible to measure several morphological characters of the stem, both gills (fig. 5d), and the cap (fig. 5b) separately for each individual sporophore.

The definitions of the measured characters are as follows:

**Area.** The area of the object.

**Perimeter.** The perimeter length of the object (Vossepoel and Smeulders, 1982).

**Shape factor.** P2a. The (perimeter-squared)/ (4*Pi*area) is a classical shape feature. P2a has a minimum value of 1.0 for a circle.

**Bending energy.** The bending energy is a measure of the amount of energy required to form the given contour (Young et al., 1974).
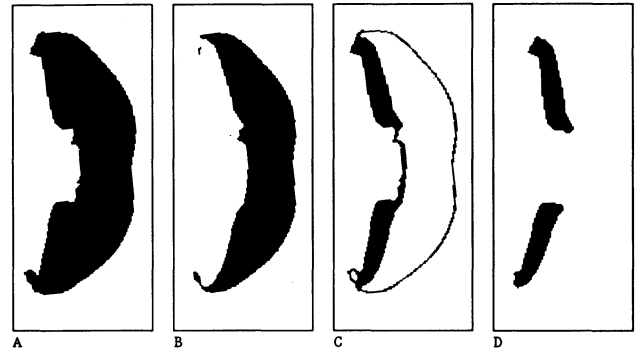


Figure 5–(a) Sporophore after first fixed threshold followed by a closing; (b) separation of cap from gills after a second, iso-data threshold; (c) the result of a XOR operation and closing; (d) erosion, propagation, and a closing shows the gills on both sides of the transverse cap.

**Rnmac.** Normalized mean absolute curvature (rnmac) is related to bending energy but it is not based on a physical interpretation such as energy (Young et al., 1974).

**Sphericity.** From every point [x(p), y(p)] on the contour we compute the radial distance R(p) to the center of mass. The maximum value of R(p) over the interval $0 \leq p \leq P$, R_max, defines the radius of the smallest circumscribed circle, and the minimum value R_min, defines the radius of the largest inscribed circle. The sphericity is then: $0.0 \leq$ sphericity = R_min/R_max $\leq 1.0$ and is 1.0 for a circle.

**Eccentricity.** Using the method of moments, an ellipse is fit to the contour of the object. The major axis (length) and minor axis (width) are determined.

**Length.** Determined as the length of the major axis of the fitting ellipse.

**Width.** Determined as the length of the minor axis of the fitting ellipse.

**Rectangle_length.** The length of the smallest enclosing rectangle (rectangle which totally surrounds the object and has a minimum area).

**Rectangle_width.** The width of the smallest enclosing rectangle.

These 11 characters were calculated for the four parts (cap, two gills, and the stem) of the sporophore, thus yielding a total of 44 measurements for each individual sporophore. These measurements could than be examined for their use for discriminating between mushroom cultivars.

In order to check if an object in an image was correctly classified (cap, gill, or stem) the following decision rules were applied. For the cap, a minimum perimeter of 40 mm and a maximum perimeter of 800 mm were set. For the gills, the minimum perimeter was set to 12 mm and the maximum perimeter was set to 80 mm. For the stem, the minimum perimeter was set to 20 mm and the maximum perimeter was set to 500 mm. The number of objects satisfying these constraints were counted. If the number differed from the expected number (one for stem and cap, two for gills) that image was tagged and an interactive analysis was performed afterwards. This was necessary in only 2 of the 460 images.

## RESULTS

One value was calculated per plot by averaging the measurements of 8 caps, 8 stems, and 16 gills. These plot

## TABLE 1. Variance ratios (V.R.) of the cultivar mean squares divided by the mean square error for all characters examined. The significance (sing.) level is indicated as follows: *: sign-level < 0.001; †: sign-level ≤ 0.05; ‡: sign-level > 0.05; §: sign. level < 0.01. Number of degrees of freedom for the numerator is 8, for the denominator 18.

| Character | Cap V.R. | Cap Sign. | Gills V.R. | Gills Sign. | Stem V.R. | Stem Sign. |
|---|---|---|---|---|---|---|
| Area | 11.63 | · * | 3.23 | · † | 0.83 | · ‡ |
| Perimeter | 7.75 | · * | 2.22 | · ‡ | 0.92 | · ‡ |
| Shape-factor | 2.67 | · † | 1.27 | · ‡ | 1.59 | · ‡ |
| Bending energy | 4.13 | · § | 0.71 | · ‡ | 0.42 | · ‡ |
| RNMAC | 4.05 | · § | 1.27 | · ‡ | 0.48 | · ‡ |
| Sphericity | 5.04 | · § | 2.10 | · ‡ | 0.82 | · ‡ |
| Eccentricity | 9.10 | · * | 13.01 | · * | 0.98 | · ‡ |
| Length | 6.43 | · * | 3.93 | · § | 1.18 | · ‡ |
| Width | 16.31 | · * | 7.76 | · * | 0.52 | · ‡ |
| R-length | 3.79 | · § | 4.01 | · § | 1.07 | · ‡ |
| R-width | 17.54 | · * | 7.56 | · * | 0.70 | · ‡ |

| | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|
| 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' | | | | | | | | | - |

Figure 6–Distinction of nine cultivars of mushroom with a 1% significance level; 2-5 repetitions per cultivar, eight sporophores per repetition. * = significantly different — = not significantly different.

means were analyzed with a univariate two-way analysis of variance (ANOVA) according to the structure of the experiment, i.e., with a cultivar stratum and a replication stratum. The cultivar∗replication mean square was used as an estimate of the variance. The effectiveness of a character for discriminating cultivars can be expressed by the cultivars mean square divided by the variance (i.e., variance ratio, Table 1). The significance level of the variance ratios is also indicated in this table. The characters were ordered according to their variance ratio and only the 15 characters with the highest variance ratio were selected. For each of these characters tables with the pairwise differences between cultivars and their probability were calculated. Among the characters with the highest variance ratio, only those characters which showed an additional contribution to the discrimination of the cultivars were selected. The finally selected characters were: area, shape-factor, and eccentricity of the cap and the shape-factor of the gills. From the combination of these four characters, it was concluded that, in 30 of 36 pairwise comparisons, the cultivars were significantly different at the 1% probability level using univariate student t-test (fig. 6).

This result proved the effectiveness of the applied image analysis techniques. The result, as shown in figure 6, was not possible with the current hand measurements—especially the shape factor of the cap and the gills, which are difficult, if not impossible, to obtain using hand measurements.

## CONCLUSIONS

Mushrooms, as most fungi, have a limited number of manually assessable morphological characters. In the case of mushrooms, it was important to assess other characters than what are currently obtainable using manual methods (Neut, 1991). With image analysis, more characters can be obtained objectively and in a fast, time-saving way. It was possible, with only the four selected characters, to distinguish 80% of the cultivars used in this experiment. Note that three out of four characters are size-independent. This result stands in large contrast with the manually assessed data with which only a grouping of similar cultivars is possible.

Considering the limited nature of information contained in a two-dimensional binary image, the results provide positive support for the capabilities of assessing morphological data with image analysis techniques. The authors aim to widen the application of image analysis in order to increase the number of botanical characters that can be assessed for various botanical objects.

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