

# Application of image analysis in plant pathology and forest protection

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**ABSTRACT:** By means of computer-based image analysis it was possible to accelerate and automate a number of originally time-consuming activities in plant pathology and forest protection. In 1997–2005, image analysis was applied to the measuring of the size of colonies of fungi *in vitro*, feeding mark area, mass measuring of the size of spores and other microscopic objects, assessment of the progress of rot in a stem etc. The paper brings particular examples of the application of image analysis including methodical procedures and examples of research results obtained using LUCIA G and QUICK PHOTO MICRO programs.

**Keywords:** image analysis; methods; plant pathology; area of colonies; LUCIA G; QUICK PHOTO MICRO

Image analysis provides a broad spectrum of possibilities for the automation and specification of a number of activities in plant pathology and forest protection. It facilitates the precise and quick evaluation of parameters of a studied object. An advantage of the method consists in the possibility to separate evaluation and measurement of the object from the actual acquisition of primary data, viz. most frequently an image in a digital form. Present digital technologies offer a trouble-free acquisition of images of corresponding resolution just in the field. In the computer, it is possible to process all basic parameters such as object dimensions, areas, statistical evaluation of parameters, determination of the size of defects, etc. All these requirements can be met at high exactness given by the scale accuracy or electronic information on the distance of a studied object, focal distance of object lens, etc. For the processing of images under a microscope or a magnifying glass it is possible to use supplied software for the evaluation of object dimensions in a microscopic field. Thus, it is possible to measure exactly both dimensions and area of spores, damaged parts of tissues, etc. Although image analysis is a standard tool used in all biological disciplines

since the beginning of the 90s, it has not been virtually used in forest protection and plant pathology in the Czech Republic yet. Thus, examples of implemented studies had to be searched in world literature until recently. For example, it is possible to cite the papers of THOMAS (1992), REICHL et al. (1992), YANG et al. (1996), COX et al. (1998) or PARK et al. (1999). Image analysis was primarily used for the determination of the area of pellets (REICHL et al. 1992; COX, THOMAS 1992), of geometric characteristics of mycelium (O'SHEA, WALSH 1996) or cell dimensions (PACKER et al. 1992). In the course of 1997–2005, possibilities of using the image analysis for phytopathology and forest protection purposes were repeatedly tested at our workplace. The aim of this paper is to present examples of these applications, their methodical procedures and examples of actual research results.

## MATERIAL AND METHODS

### Acquisition of image material

To acquire the image material, photographs from digital cameras were used with an inserted scale

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for image processing in LUCIA G program or with stored internal data of Olympus digital cameras on exposure in the case of processing by a variant QUICK PHOTO MICRO program.

### **Processing in LUCIA G program**

LUCIA G is a system of image analyses which processes and analyses digital images, facilitates the communication between a scanning device, scanning (grabbing) card and a computer. Output information of these three devices consists of an image prepared for further processing. Through the processing we can obtain colour and morphological information on objects under study or a suitably prepared image for documentation. LUCIA G program uses its own format “\*.lim” to store the image information. The format is created by loss-free fractal compression storing user-defined information on a scanned picture and can be used for installing an additional modulus in common applications. Moreover, the image can be stored right in LUCIA G program to a standard image format, such as \*.bmp, \*.tif, \*.rle, etc.

One of many functions of the program is the analysis of image stages (colour components). Among others, it is also possible to calculate their area. The actual procedure of processing the images can be divided into several steps:

#### ***Image calibration***

Photographs taken with a digital camera were opened in LUCIA G program. On the basis of the depicted scale real dimensions were assigned to images, i.e. a calibration constant was put into them and the images were stored in “\*.lim” format. This format makes it possible to immediately evocate the calibration constant at the subsequent opening of the image and the system sets the actual dimensions of depicted objects regardless of the initial image scale (thus, microscopic objects as well as areas of several hectares can be depicted).

#### ***Delimitation of the object area (colony, rot, etc.)***

The object area definition (delimited colour phases) was most often carried out semi-automatically (however, a manual procedure is also possible). The principle of this procedure is that the image analysis program is able to identify colour values (RGB components) of all pixels of an image. Thus, it can mark all pixels (the smallest particles of an image) showing the same or similar RGB values. This procedure is termed thresholding. It takes place most often manually under the supervision

of an operator, however, it is possible to automate it (particularly in the analysis of a higher number of images with similar colour parameters) using a special macro.

The result of thresholding is a monochromatic binary image delimiting all pixels of an image complying with the condition given in thresholding. The binary image is mostly further adjusted by means of image analysis based on mathematical morphology. A final image delimiting the actual and often very irregular area of a colony is stored together with the calibration constant of an original image (it shows the same dimensions as the original image in \*.lim format).

### ***Measurement and acquisition of results***

The binary image generated by the procedure mentioned above is then measured by means of image analysis (measurement of areas). And again, it is possible to determine the way of measurement and various specifications, to exclude or, on the contrary, to include certain objects, etc. The measurement is carried out very quickly and if necessary also other characteristics can be measured within the process (length, width, angles, circumference, circularity, eccentricity and other special characteristics commonly used in the analysis and processing of the image). Resultant data are transferred to MS Excel and stored in the form of data files.

### **Processing the image in QUICK PHOTO MICRO program**

The procedure and principles of image processing are similar like in LUCIA program. The QUICK PHOTO MICRO program is devised to record a digital image above all from microscopes equipped with digital cameras OLYMPUS CAMEDIA. A live image is transferred from a microscope right to the PC monitor and makes easier focussing and correct exposition of the image possible. After scanning and storing data in the PC the following functions are available: measurements of lengths (line segment, broken line), inserting a calibrated scale, measurements of circumferences and areas (ellipse, rectangle, polygonal area), measurements of angles, counting of objects and analysis of phases. Actual results are recorded in tables with a possibility to supplement notes and for further processing in MS Excel. The following possibilities are also available: image rotation and turning, trimming (dimensions can be defined in pixels,  $\mu\text{m}$  or mm), adjustment of brightness and contrast, adjustment of colour balance, use of filters (focusing, defocusing, the

scale of grey), marking interesting parts, inserting legends, etc.

All objects inserted into an image are placed into a separate layer "above the image" and can be modified, covered or deleted at any time. The program makes it possible to operate with more images simultaneously. For the purpose of easy observation it is possible to place them side by side and so to create a simple presentation. In the QUICK PHOTO MICRO program, it is possible to create protocols from measurements in Microsoft Word format. It is also possible to use templates of proposals of particular protocols.

#### Methodical procedures used in demonstrations of the image analysis application

Photographs which were the basic material for the demonstrated analyses of images were taken with a digital camera.

**Measurements of the rate of growth of colonies of fungi** – By means of image analysis through the LUCIA G program growth curves were studied of *Armillaria* spp. colonies which are characterized by the colony irregularity. Thus, the area measurement is the only expression of growth rate. The colonies were obtained by the standard method of cultivation.

**Measurements of the size of microscopic objects** – By means of image analysis through QUICK PHOTO MICRO 2.2 program dimensions were examined of *Sphaeropsis sapinea* conidia from images taken with a digital OLYMPUS camera in connection with the OLYMPUS SZX 12 microscope.

**Measurements of the size of feeding marks of a cambioxylophagous insect** – By means of the LUCIA G program dimensions of galleries of a cambioxylophagous insect were measured in usual digital images.

**Measurements of the area of rot** – In selected spruce stands of young and medium age, in the

Forest District Proklest (the Křtiny Training Forest Enterprise Masaryk Forest), sample trees with mensurational quantities approaching the mean stem values were felled. The sample trees were cut to one-metre sections until the rot caused by *Stereum sanguinolentum* was evident at the end of a section. In case that the rot was not evident at the section end at all, the section was further divided in such a way that the height of rot in the stem be determined as correctly as possible (from the place of the stem damage). A cut was also carried out in the place of a wound after deer barking or browsing. Cutting areas (section ends and a cut in the place of wound) were photographed (with a respective scale and sample tree/section number). In the LUCIA G program, areas of rot were measured on particular section ends, volumes of wood affected by rot were calculated as well as rates of the horizontal spread of rot on a cut in the place of wound after deer barking.

## RESULTS AND DISCUSSION

### Measuring the growth rate of colonies of fungi

Areas of colonies of *Armillaria* spp. (Fig. 1.) and some other growth characteristics were measured. Average values were characterized by mean deviation and standard deviation. The growth of cultures at various temperatures was presented in diagrams and tables (Table 1). The former measurements of growth characteristics of fungi were based above all on measurements of the diameter of colonies. The quantity is difficult to measure in some species of fungi creating rhizomorphs and secondary colonies forming individually upper to rhizomorphs. Using the computer-based image analysis it is possible to measure a minimum and maximum diameter quite easily, and to measure the areas of colonies very easily and accurately on the basis of contrast colour differences between the colony and its background. Thus, image analysis made it possible to express the

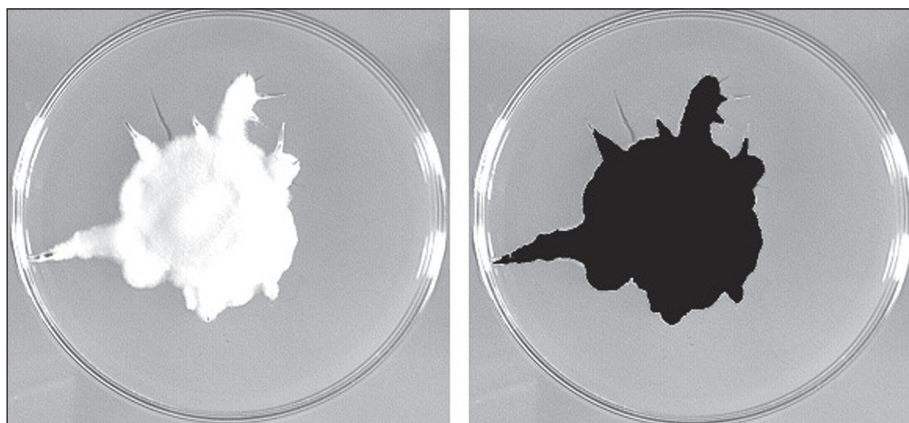
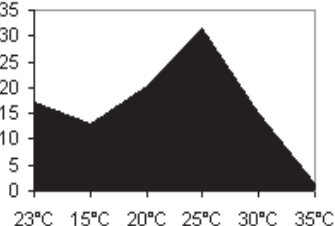


Fig. 1. Thresholding of *Armillaria* spp. colony surface. On the right cleaned binary image. Processed in LUCIA G

Table 1. Evaluation of the effect of temperature on the growth of *Armillaria* mycelium in the malt extract agar. Processed in LUCIA G

	Surface of colony (cm <sup>2</sup> ) at temperature (°C)						Colony growth
	23	15	20	25	30	35	
Species	aver.	aver.	aver.	aver.	aver.	aver.	
Number of strains	aver. dev.	aver. dev.	aver. dev.	aver. dev.	aver. dev.	aver. dev.	
	stand. dev.	stand. dev.	stand. dev.	stand. dev.	stand. dev.	stand. dev.	
Æ	17.22	12.79	20.27	31.56	15.07	1.42	
<i>Armillaria</i>	7.27	5.53	10.46	15.47	6.53	0.92	
<i>gallica</i>	11.4	7.91	13.98	19.56	1.18	1.07	
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growth of colonies very accurately, markedly extending and specifying the present findings on *Armillaria* spp. (JANKOVSKÝ, SCHÁNĚL 1997).

By means of the QUICK PHOTO MICRO 2.2 program the growth of mycelia of a quarantine harmful organism *Cryphonectria parasitica* was monitored (JANKOVSKÝ et al. 2003; HALTOFOVÁ 2005). And again, the growth of colonies was documented in the digital form and the subsequent measurement of the colony area was done on Petri dish. The maximum and minimum diameter of a colony was also measured.

Outside the Czech Republic, image analysis was used to observe morphology, dimensions and growth of pellets of a number of groups or species of fungi and bacteria, e.g. *Streptomyces tendae* (REICHL et al. 1992), *Streptomyces fradiae* (PARK et al. 1997), *Aspergillus niger* (COX, THOMAS 1992), *Kluyveromyces marxianus* var. *marxianus* (O'SHEA, WALSH 1996) or *Mortierella alpina* (PARK et al. 1999). All papers agreed that image analysis markedly simplified and specified measurements resulting in high-quality visually effective outputs.

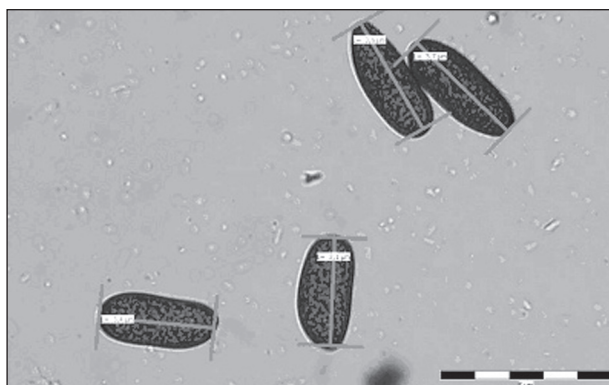


Fig. 2. The measurement of conidial length on the example of *Sphaeropsis sapinea*. Processed in QUICK PHOTO PRO application

### Measuring the size of microscopic objects

By means of QUICK PHOTO MICRO 2.2 software mentioned above, in connection with a microscope and camera it is possible to evaluate dimensions of microscopic objects such as spores. Using automated evaluation it is possible to carry out statistical analysis of the size of spores or other morphological parts of fungi. The measurement of object dimensions is markedly simplified and thus it is necessary to insert a scale into each image. We can present the measurement of conidia of *Sphaeropsis sapinea* in Fig. 2 as an example of the measurement of objects. Even there, marked simplification and specification of measurements are a resultant effect.

### Measuring the size of galleries of a cambioxylophagous insect

Areas of galleries of a cambioxylophagous insect can indicate changes in physiological processes of the insect or its activity very sensitively. The measurement of such complicated figures as galleries can be carried out by standard procedures only with difficulties. Thus, image analysis makes possible quick and accurate measurements of objects on the basis of colour differentiation of the area of feeding marks from neighbouring tissues. In new feeding marks, it is possible to carry out evaluation right from photographs. In older feeding marks, it is necessary to distinguish the area of feeding marks by a colour as demonstrated in Fig. 3 on the example of *Ips typographus* feeding traces. It is also possible to measure other important parameters such as the length of mother galleries. Thus, easy acquisition of considerable amounts of data can contribute to the dissemination of knowledge concerning the vari-



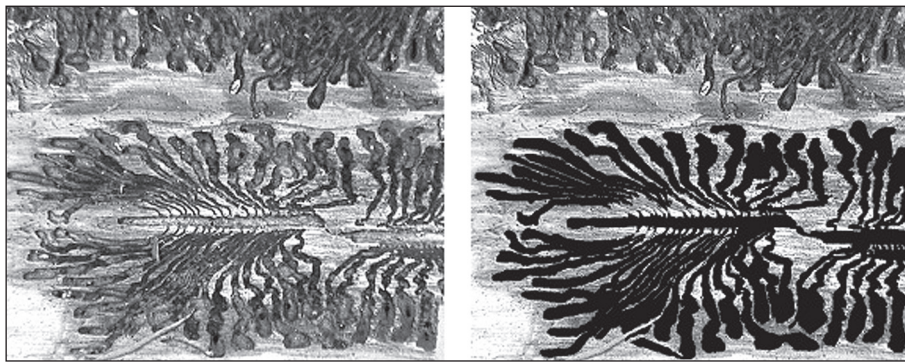


Fig. 3. The morphometric processing of bark beetle gallery. On the left – original picture (in colours), on the right final cleaned binary image, prepared for surface and length measurement. Surface of gallery – 158.6 cm<sup>2</sup>, length of mature gallery – 12.42 cm. Processed in LUCIA G

ability of dimensions of feeding marks, facilitate the determination of species, improve knowledge of the activity dynamics in particular species in relation to external conditions. The measurement of the area of galleries can also result in its quite practical use for the determination of physical properties of wood or its aesthetic values. It can be demonstrated on the identification of the area of feeding marks caused by *Coroebus undatus* and *Crematogaster scutellaris* in cork slabs (GONZALEZ-ADRADOS et al. 2000).

In addition to measurements of the area of feeding marks other possible uses are offered concerning particularly insect species causing mass outbreaks. In photographs, it is possible to evaluate defoliation of trees and on its basis to draw a conclusion about the rate of insect attack. In these applications, first it is necessary to create scales and to calibrate them carefully. Image analysis was used for the determination of attacked and unattacked trees, e.g. in the pine *Pinus contorta* attacked by bark beetles in Canada (MURTHA, WIART 1989).

#### Measurements of the area of rot

The assessment of the interface between rotten and uninfected wood with the naked eye is difficult.

The image software made it possible to stress colour phases (Fig. 4) and subsequently to determine the area of rot exactly. High-quality digital images enabled the accurate identification of a period elapsed from the damage origin caused by browsing or barking necessary for the determination of the rate of rot progress. In the monitored area of Forest District Proklesť, the rot spread in a horizontal direction (laterally) at an average rate of 35 cm<sup>2</sup>/year (from 2 to 133 cm<sup>2</sup>/year). At this rate of spread, the area of rot in the place of wound amounted to 45% on average. The data correspond to conclusions of similar research carried out in northern Europe (VASILIAUSKAS, STENLID 1998) where the average proportion of the rot-affected area in the place of cut amounted to 36.8%. ČERNÝ (1976) reported the values of horizontal spread about 20 cm<sup>2</sup>/year for *Stereum sanguinolentum* and other rots and the same data were given by VASILIAUSKAS and STENLID (1998) from Lithuania.

The rot destroyed 0–66.8% of the wood volume of the stem sections with the occurrence of rot, on average 42.62%, mean values of particular sections are given in Fig. 5. In absolute values, the volume of the rot-destroyed stem amounted to 0.08 m<sup>3</sup> on average. Another potential use of the demonstrated

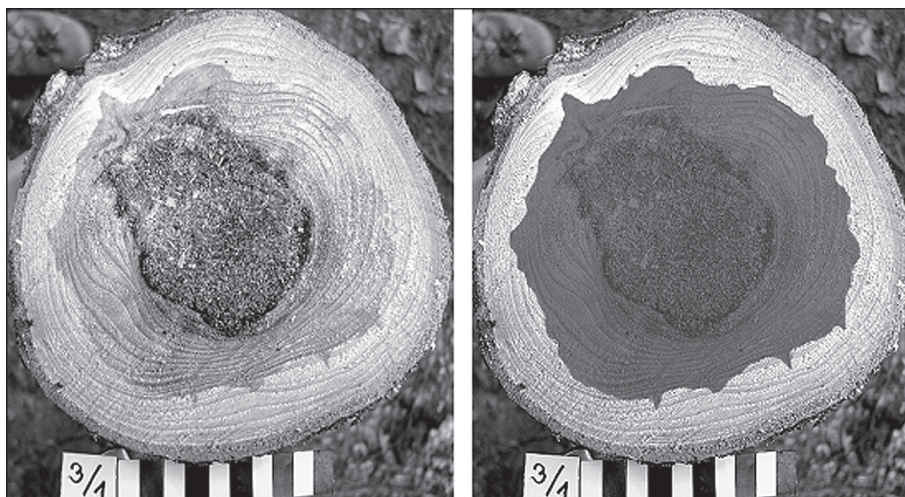


Fig. 4. The measurement of the surface of rotten wood in the cutting area. Section 3/1 – the surface of rotten wood is 183.50 cm<sup>2</sup>, the surface of the cutting area is 318.50 cm<sup>2</sup>. Processed in LUCIA G

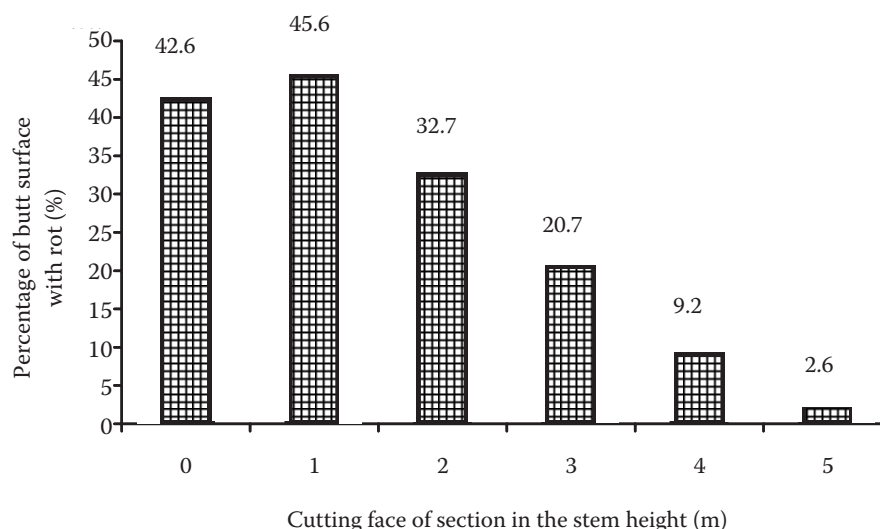


Fig. 5. The average of rot surface on the cutting face. The mean cutting face surface affected by decay in a percentage relationship to all sample trees. Processed in LUCIA G (ČERMÁK et al. 2004)

software for monitoring damage caused by barking and browsing can be oriented e.g. to the differentiation of the proportion of particular phases of rot in the place of cut or the measurement of wound area after deer barking.

## CONCLUSION

Using the image analysis it was possible to automate a number of originally lengthy activities – it is possible to compare changes in studied objects in longer time periods and to process data from a “time-collecting” camera with sufficient resolution. Image analysis was applied to the measuring of the size of colonies of fungi *in vitro*, mass measuring of the size of spores, determination of the area of feeding marks of a cambioxylophagous insect or assessment of the lateral progress of rot in the tree stem. Our paper indicates possibilities of this use including methodical procedures. A basic condition for the use of image analysis is the sufficient differentiation of the studied object (or its part) from its vicinity and a possibility of thresholding colour differences. A contrast in boundaries of the object under evaluation and its vicinity is of particular importance.

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## Aplikace analýzy obrazu ve fytopatologii a ochraně lesa

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**ABSTRAKT:** Pomocí analýzy obrazu se podařilo zrychlit a zautomatizovat řadu původně zdoluhavých činností v lesnické fytopatologii a v ochraně lesů. V letech 1997–2005 byla analýza obrazu na našem pracovišti aplikována při měření velikosti kolonií hub *in vitro*, plochy požerků, hromadném měření velikosti spor a dalších mikroskopických objektů, hodnocení postupu hniloby v kmeni atd. Práce přináší konkrétní příklady aplikací analýzy obrazu včetně metodických postupů a ukázek výzkumných výsledků získaných s využitím programů LUCIA G a QUICK PHOTO MICRO.

**Klíčová slova:** analýza obrazu; metodika; lesnická fytopatologie; povrch kolonií; LUCIA G; QUICK PHOTO MICRO

Analýza obrazu poskytuje široké spektrum možností pro automatizaci a zpřesnění řady činností ve fytopatologii a ochraně lesa. Umožňuje přesné a rychlé vyhodnocení parametrů sledovaného objektu. S výhodou byla aplikována při měření velikosti kolonií hub *in vitro*, hromadném měření velikosti spor, stanovení plochy požerku kambioxylofágního hmyzu či hodnocení laterálního postupu hniloby v kmeni. Příspěvek předkládá možnosti těchto aplikací včetně metodických postupů při využití softwaru LUCIA G či QUICK PHOTO MICRO.

S využitím analýzy obrazu je možné relativně jednoduše změřit minimální a maximální průměr kolonie hub v kulturách *in vitro* a také jejich plochy – na základě kontrastních barevných rozdílů mezi kolonií a pozadím. Analýza obrazu umožňuje velmi přesné a graficky efektní vyjádření růstu kolonií.

Pomocí automatizovaného vyhodnocení fotografií pořízených přes mikroskop lze provést statistickou analýzu velikosti spor či jiných morfologických (mikroskopických) částí hub. Měření velikosti objektů se přitom výrazně nejen zpřesňuje, ale také zjednodušuje, není totiž nutné do každého obrazu vkládat měřítko.

Aplikací analýzy obrazu lze rychle a přesně měřit požerky kambioxylofágního hmyzu (plocha, délka matečné chodby) – na základě barevného odlišení jejich plochy od okolních pletiv. U čerstvých požerků je možné provést vyhodnocení přímo z fotografií, u starších požerků je nutné plochu požerků odlišit barvou.

Kvalitní digitální fotografie umožňují díky softwaru LUCIA G přesné určení doby uplynulé od vzniku poškození ohryzem či loupáním (spočítání letokruhů), zvýraznění odpovídající barevné fáze a následně tak přesné určení plochy hniloby na dané řezné ploše.

Další možné využití demonstrovaného softwaru může směřovat například k rozlišování podílu jednotlivých fází hniloby na řezu, k měření velikos-

ti rány vzniklé loupáním či k hodnocení defoliace stromu.

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