5 Deligne J., Quennedy, A., and Blum, M. S., The enemies and defence mechanisms of termites, in: Social Insects. Ed. H. R. Hermann. Academic Press, 1982.
6 Prestwich, G. D., A. Rev. Ent. *29* (1984) 201.
7 Jones, G., Insectes soc. *29* (1982) 297.
8 Prestwich, G. D., A. Rev. ecol. Syst. *14* (1983) 287.
9 Woodward, R. B., Sondheimer, F., Taub, D., Heusler, K., and McLamore, W. M., J. Am. chem. Soc. *74* (1952) 4223.
10 Barbier, M., Introduction to Chemical Ecology. Longman 1979.
11 Blum, M. S., Chemical Defences of Arthropod. Academic Press, 1981.
12 Wood, W. F., Truckenbrodt, W., and Meinwald, J., Ann. ent. Soc. Am. *68* (1975) 395.
13 Wood, W. F., Ann. ent. Soc. Am. *67* (1974) 988.
14 Wood, W. F., Shepherd, J., Chong, B., and Meinwald, J., Nature *253* (1975) 625.
15 Meinwald, J., Prestwich, G. D., Nakanishi, K., and Kubo, I., Science *199* (1978) 1167.

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Air ventilation in nests of two African stingless bees *Trigona denoiti* and *Trigona gribodoi*

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Summary. The ground nesting *Trigona denoiti* as well as the arboreal nesting *T. gribodoi* have nest cavities with only a single narrow entrance tube. For air exchange in the nest to occur, the bees have to ventilate the nest actively. As a consequence the air is exchanged in a similar way as in vertebrates with lungs. Phases of inspiration follow phases of expiration ('breathing' frequency in the range of 2–3 breaths/min) and tidal volumes range from 0.63 ± 0.24 ml in *T. denoiti* to 1.13 ± 0.32 in *T. gribodoi*. Ventilation during night time was strongly reduced.
Key words. Respiration; social behavior; *Trigona denoiti; Trigona gribodoi.*

The regulation of the nest climate is a crucial problem for all eusocial insects. The brood is in many species highly sensitive to temperature extremes and quickly dies if the nest humidity is not regulated. Various strategies for nest climate control have been developed within the social insects, ranging from the transport of brood within the nest to warmer or cool areas (e.g. in ant nests[1,2]) to the highly complex regulation of nest climate in honeybees (*Apis mellifera*).

Though the control of nest climate in honey bees is well understood[3–7], in stingless bees data are very scarce[8]. In particular African species have been neglected, although they have interesting nest architectures which might impose specific behavioral requirements for nest climate control. A nest cavity with a single narrow entrance tube is typical for all *Trigona* species in southern Africa[9]. The stingless bees either nest in hollow trees or subterranean cavities. The cavities are coated with batumen, the typical nest material of the stingless bees. It consists of water-proof resin and is a good insulator.

T. denoiti nests 60–100 cm deep in the ground. The nest as well as the entrance tube is lined with batumen. The brood nest has a spiral structure and uses the small nest cavity most efficiently. The brood nest temperature is regulated by the bees, though a mechanism for active cooling seems to be absent[10]. A long, narrow entrance tube (1 cm diameter, more than 60 cm long) connects the nest with the outside, which may make cooling of the nest by fanning extremely difficult. The bees apparently nest at a great enough depth to achieve a stable nest climate even when the outside temperatures are high.

T. gribodoi nests in cavities of hollow trees[11]. In Transvaal they are mainly found in the Red Bush Willow (*Combretum apiculatum*). They also have only a single narrow nest entrance tube (0.7 cm diameter, 10–20 cm long). The nest does not overheat because of the shade and insulation provided by the tree. Also in this species no active cooling has been observed, though they actively produce heat if the temperature drops below 31 °C (B. Weissenbacher, unpublished data).

Although a small nest entrance with a long connective tube to the nest is effective in reducing water loss and buffering against ambient temperature variation, it may create substantial problems for the regulation of the concentration of the respiratory gases within the nest. The narrow entrance tube allows no flow-through system for fresh air as found in termite mounds[12,13]. Unless the bees can withstand extremely low oxygen levels in the nest, they have need for a ventilatory system which exchanges old stale air with fresh air.

Southwick and Moritz[14] showed that colonies of honeybees are able to control the concentration of the respiratory gases even if there is only a single narrow entrance tube to the hive. The honeybee workers actively fanned stale air out of the hive, producing a pattern similar to that of breathing of mammals, with an inspiration and expiration phase. Nests of honeybees, however, very often have a large entrance or even multiple entrances to the nest cavity and it is believed that in most cases the honeybee workers ventilate their nest in such a way that there is a continuous unidirectional airflow through the nest. The observed phenomenon therefore might be of small relevance in natural colonies of honeybees.

In the African stingless bees, on the contrary, the single entrance tube is definitely a physiological phenomenon. Since there is only one connection to the outside world, and the nest cavity is lined with airtight batumen, the bees should generate a type of 'colony breathing', similar to that found in the experimental setup with honeybees. In this paper we study how air exchange and the ventilatory system in ground nesting and arboreal stingless bees operates.

Materials and methods. Two nests of each species were localized in the Lapalala Game Reserve, Transvaal, South Africa. A copper/constantane thermocouple connected to a recorder, was placed at different levels into the entrance hole, to document the temperature gradient from the outside air to the nest. Thereafter the thermocouples were positioned 10 cm inside the entrance tube and the changes in temperature were recorded for at least 20 min (fig. 1). During the day, when the ground surface temperature exceeded 60 °C, an increase in

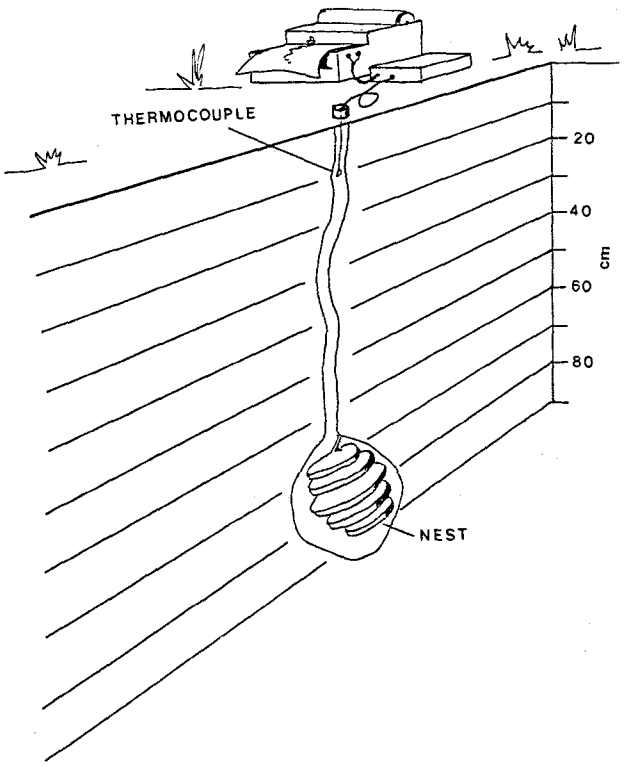

Figure 1. Diagram of a nest of *T. denoiti* and positioning of the thermocouple in the entrance tube. Entrance tube and nest cavity are lined with batumen.

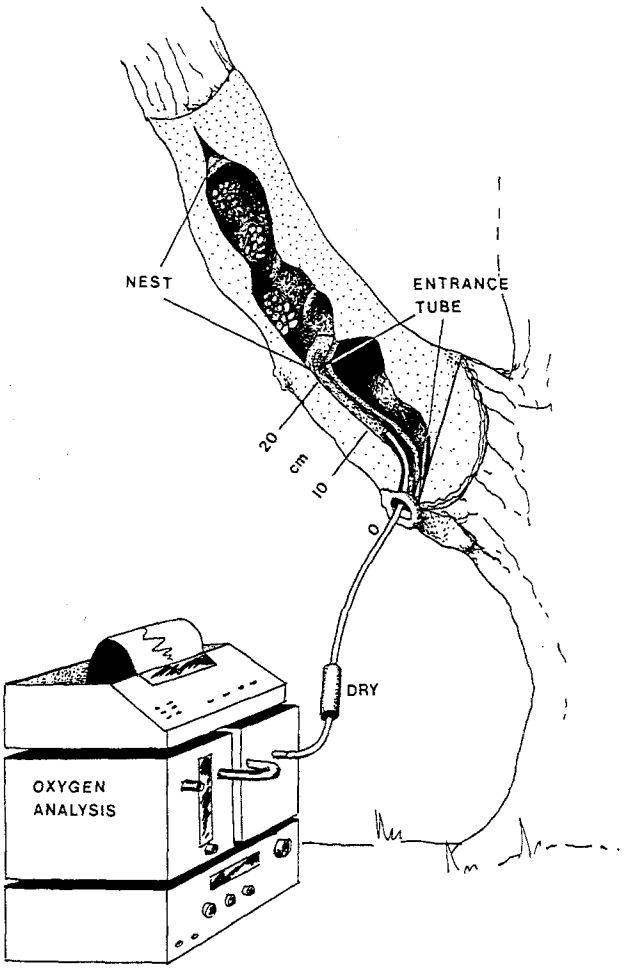

Figure 2. Diagram of a nest of *T. gribodoi* and positioning of the sampling tube for the oxygen analysis. A 10–20 cm entrance tube is built inside the cavity. The portion of the cavity within the branch that is used by the bees, is sealed with batumen.

temperature revealed the influx of hot outside air. A temperature drop in the entrance tube showed expiration of cooler but stale inside air.

In addition to the thermal measurements, the oxygen concentrations in the entrance tube were measured. A small polythene tube (2 mm outer diameter) was carefully pushed into the entrance hole (fig. 2). The tube was connected to a pump, which forced the air after drying at a low flow of 15 ml/min through an oxygen analyzer (S3A/II, Ametec, Applied Electrochemistry). An oxygen concentration profile over the length of the entrance tube was taken. Short-term changes in the oxygen concentration were measured at a fix level (10 cm) for at least 20 min. An increase in the oxygen concentration indicated the influx of fresh air. The data were recorded on a HP 3380A integrator for further documentation. A portable generator supplied the necessary power for the electronic equipment in the field.

The ground nesting *T. denoiti* was also tested during the night (21.00–23.30 h) in order to reveal differences in the ventilatory behavior during possible resting periods.

Results. Trigona denoiti. The temperatures within the two nests in the study were very similar even though outside temperatures differed substantially (fig. 3). At an outside temperature of 60.9 °C the nest temperature was 32.0 °C. The nest temperature stayed constant during the night, even though the outside temperature dropped by more than 30 °C The thermocouples were placed at a level of 10 cm and the recordings showed regular oscillatory temperature changes in both colonies, one of which is represented in figure 4. The 'breathing' frequencies were similar in both nests with 2.35 ± 0.20 breaths/min (bpm; n = 28) and 2.41 ± 0.21 bpm (n = 26). Using the regression function from the temperature profile, we could estimate the actual volume of air moved during ventilation. This allowed us to estimate tidal volumes which averaged at 0.83 ± 0.1 ml (n = 54). A min-

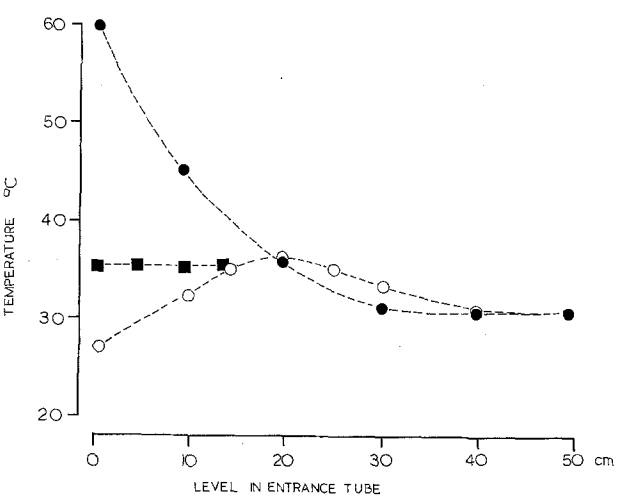

Figure 3. Typical temperature gradient in the entrance tube of a *T. denoiti* (day: ●; night: ○) and a *T. gribodoi* nest (■).

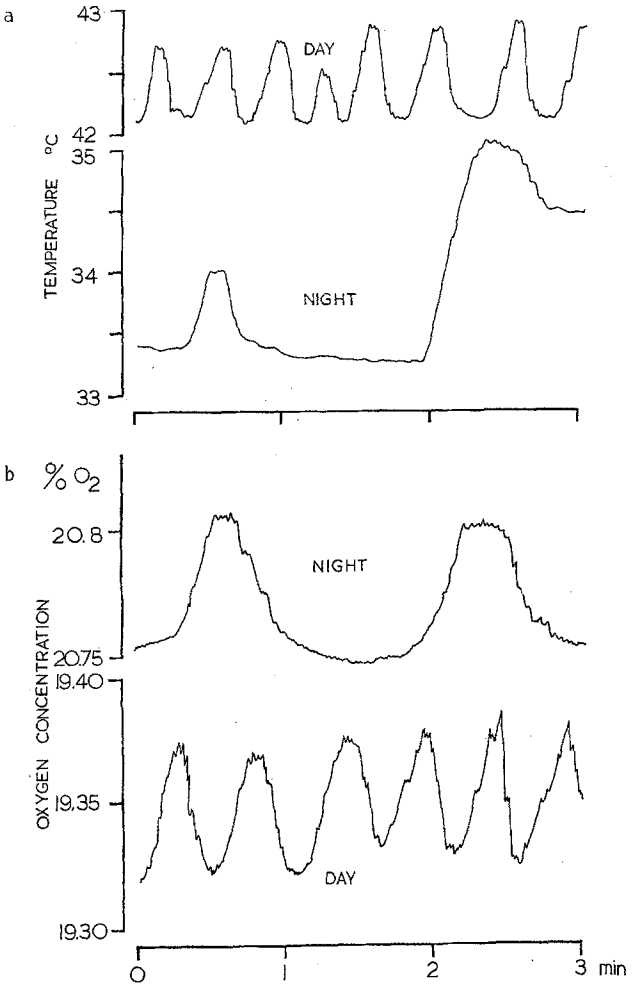

Figure 4. Typical respiratory oscillations during day and night time, determined by *a*) temperature and *b*) oxygen concentration fluctuations in the entrance tube (10 cm level) in a *T. denoiti* nest (no simultaneous recordings!).

Average respiratory parameters ± SE of nest of *T. denoiti* and *T. gribodoi* during day and night time. n = number of 'breaths' analyzed.

	T. denoiti Day (n = 54)	Night (n = 24)	*T. gribodoi* Day (n = 58)
Breathing frequency	2.38 ± 0.20	0.62 ± 0.05	5.45 ± 0.36
Tidal volume [ml]	0.63 ± 0.10	1.14 ± 0.38	1.13 ± 0.20
Maximal tidal volume	0.85	3.20	1.51
Minute volume [ml/min]	1.98 ± 0.25	0.79 ± 0.41	6.16 ± 0.38

volume of 1.98 ± 0.25 ml was estimated with this technique. The oxygen measurements revealed similar results to those from the thermal measurements. A gradient in the oxygen concentration (fig. 5) from fresh air (20.94%–18.5%) at the 57 cm level during daytime (19.99% at night) enabled us to estimate tidal volumes and respiratory frequencies. There was no significant difference between the estimates from the thermocouple measurements and the oxygen concentration readings and the pooled data from both techniques is presented in the table.

The measurements taken at night (21.00–23.00 h) were significantly different from the daytime data (table). In the initial phase shortly after 21.00 h some tidal volumes were extremely high (more than 3 ml). Later the tidal volume decreased, but was still higher than during the day. However,

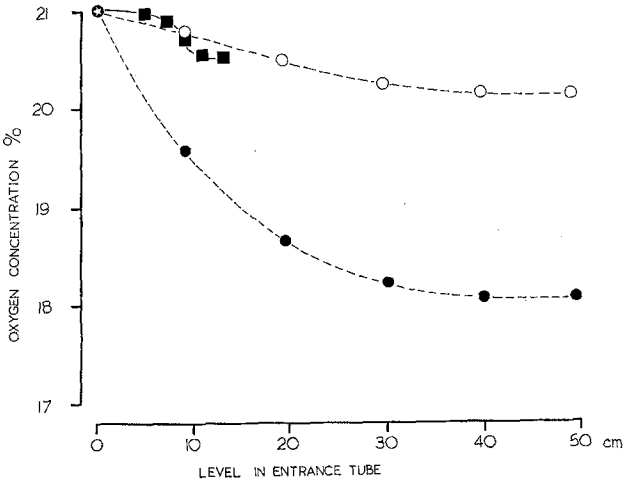

Figure 5. Gradient of oxygen concentration in the entrance tube of a *T. denoiti* (day: ●; night: ○) and a *T. gribodoi* nest (■).

the breathing frequency was reduced which resulted in significantly smaller min-volumes (0.79 ± 0.11 ml).

Trigona gribodoi. The temperature measurements taken at the tree nesting colonies revealed only a very weak temperature gradient from the inside to the outside air (fig. 3). The temperature of the air 10 cm inside the entrance tube, recorded in 5-s intervals, was extremely constant 35.04 ± 0.01 °C whereas the outside temperature in the same time period showed a significantly larger variance 36.38 ± 0.33 °C ($F_{71,71} = 32.11$; $p < 0.01$). Due to the small gradient, the thermocouples could not be used to reveal any ventilation of the nest.

The oxygen concentration in the nest entrance (fig. 5), however, had a steep gradient. This allowed for the estimation of the respiratory parameters (table) from the oscillation of oxygen contents in the nest entrance (fig. 6). Since the breathing frequency was significantly higher in *T. gribodoi* than in *T. denoiti* the resulting min-volume (6.2 ml) of the tree nests is almost four times as high in the ground nests.

Discussion. Apparently both *Trigona* species actively ventilate their nests. The tidal air ventilation has little to do with the breathing of individual workers in the nest. It is unlikely that all individuals of the nest coordinate their individual breathing. Like in honeybee colonies, the colonial breathing in stingless bees is most probably a result of workers fanning in the nest cavity. In honeybees the workers actively fan stale air outward, which creates a small decrease of pressure inside the fix nest cavity. The influx of fresh air is passive and

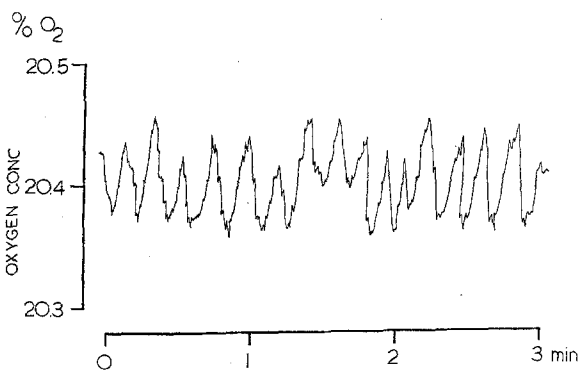

Figure 6. Typical respiratory oscillations in a *T. gribodoi* nest determined by fluctuations of the oxygen concentration in the entrance tube.

equilibrates the pressure difference between inside and outside the nest. The moved air volumes in both *Trigona* species are small in comparison to honeybee colonies, but in the light of the minute size of the stingless bees and their nests the volumes may not be so small. Since the ventilation is due to fanning of workers, the wing surface area may be an important parameter (among others) for the determination of tidal and minute volumes. If we relate the min-volumes of the stingless bee colonies to wing surface area, we obtain results in the same range than previously found for honeybee colonies[12] (min volume/wing surface area: *Apis mellifera:* wing surface approx. 15 mm^2, 8 ml/min/mm^2; *T. denoiti:* wing surface approx. 1.52 mm^2 0.55 ml/min/mm^2; *T. gribodoi:* wing surface approx. 1.42 mm^2, 4.4 ml/min/mm^2). If we look at the nest volumes we see that it takes a long period of time to exchange the complete gas volume in *T. denoiti*. The ventilatory activity in *T. gribodoi* exchanges the total nest volume (< 500 ml) in less than 1.5 h. This rate may be sufficient to prevent the accumulation of CO_2 in the colony. Strong nests of *T. gribodoi* consist of only 700–800 bees and a few hundred brood cells[11]. Indeed the recorded O_2 concentrations were high during the day. However, nests of *T. denoiti* have a gas volume of approx. 1000 ml[10] and it would take 7–8 h to exchange this volume at the observed breathing rates. Colonies consist of more than 10,000 bees, and of 10–14 brood combs each containing about 500 brood cells[10]. Even considering that the metabolic activity of bees and brood inside the nest is very low, the minute daytime ventilation should cause an accumulation of CO_2 in the nest cavity. This is supported by the very low levels of oxygen during the day, which most likely result from a high CO_2 concentration in the nest. During our recordings at night, the oxygen level was substantially higher than during the day. Apparently the bees had flushed the nest before we started our night-time recordings. Initially we could observe large tidal volumes resembling yawns in mammals, which became rare once the oxygen concentration was higher than 20.7%. It would need only 1–2 h of ventilation with those large tidal volumes to exchange the complete nest volume. Like in honeybees the activity of the bees was low during the night. The nest temperature which was constant day and night may mainly be an indication for the insulative value of the soil rather than metabolic activity of the bees[10].

The colonial breathing pattern discovered in stingless bees is very much the same as in honeybee colonies housed in airtight containers with a single entrance hole only. The breathing frequencies of honeybee colonies were in the same range (2.9 ± 0.84 bpm) and they also reduce the ventilatory activity during the night substantially. Min-volumes were about 10% of the daytime values, a reduction more drastic than in *T. denoiti* nests (39%).

The small tidal volumes of *T. denoiti* during daytime prevents the nest from overheating. The cool air stays in the nest and only as little as possible hot fresh air is introduced. During the night, when the outside air is cool, the tidal volumes are significantly higher.

The higher minute volume in the arboreal *T. gribodoi* may also result from the less severe temperature conditions in comparison to the ground nesting species. Ambient temperatures for *T. denoiti* nest entrances were as high as 60° C at the soil surface, whereas the outside air in the shade of the tree was only 36° C. The influx of fresh air is a small thermal danger to the brood for *T. gribodoi*. The brood is particularly sensitive to high nest temperatures and dies in the case of *T. denoiti* at 35.5° C, which is just 1° C above the optimal brood nest temperature[8]. This may also explain why *T. denoiti* nests ventilated at night-time with relatively large tidal volumes. Because of the low outside temperature there was no danger of overheating the brood.

In general we found that under physiological conditions social insects are able to control the concentration of their respiratory gases in a similar fashion to that of mammalian organisms. It seems that 'colonial breathing', originally found in honeybees, is a general phenomenon for social bees with nests with single entrance tubes. Colonial breathing is yet another physiological trait of colonies of social bees similar to mammalian organisms and may give further support to the physiological superorganism model[15] of colonies of social insects.

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1 Gösswald, K., Z. wiss. Zool. *151* (1938) 337.
2 Schmidt, G. H., in: Sozialpolymorphismus bei Insekten, p. 404. Ed. G. H. Schmidt. Wissenschaftliche Verlagsgesellschaft, 1974.
3 Heinrich, B., in: Experimental Behavioural Ecology, p. 393. Eds B. Hölldobler and M. Lindauer. Gustav Fischer, 1985.
4 Himmer, A., Erlanger Jb. Bienenk. *5* (1927) 1.
5 Lindauer, M., Z. vergl. Physiol. *36* (1954) 391.
6 Simpson, J., Science *133* (1961) 1327.
7 Southwick, E. E., J. comp. Physiol. *156 B* (1985) 143.
8 Michener, C. D., The Social Behavior of the Bees. Harvard Press, 1974.
9 Smith, F. G., Proc. Roy. ent. Soc. *29* (1954) 62.
10 Fletcher, D. J. C., and Crewe, R. M., J. ent. Soc. South Africa *44* (1981) 183.
11 Bassindale, R., Proc. zool. Soc. Lond. *125* (1952) 49.
12 Lüscher, M., Sci. Am. *205* (1961) 138.
13 Lüscher, M., Acta trop. *12* (1955) 289.
14 Southwick, E. E., and Moritz, R. F. A., J. Insect Physiol. *33* (1987) 623.
15 Wheeler, W. M., J. Morph. *22* (1911) 307.

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Specific effects of diterpene resin acids on spore germination of ectomycorrhizal basidiomycetes

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Summary. Six diterpene resin acids out of eight tested possess the capacity to induce spore germination in all ten Swedish and American species of *Suillus* tested. Species from other genera did not respond. A method for isolation of homokaryons (monosporous mycelia) of *Suillus* species is described.
Key words. Diterpene resin acids; ectomycorrhizal basidiomycetes; spore germination; *Suillus*.