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PRODUCTION AND DISPERSAL OF BASIDIOSPORES OF *Ganoderma applanatum* IN JAPAN

日本におけるコフキサルノコシカケの担子胞子の生産と散布

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL OF NATURAL SCINCE AND TECHNOLOGY OF KANAZAWA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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Dissertation

Production and dispersal of basidiospores of *Ganoderma applanatum* in Japan

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ABSTRACT

Discharge, dispersal, and germination of spores are three important things for fungal to producing the new generation. The genus of Ganoderma (Fungi) has been known to produce an enormous number of spores with a thick outer wall. *Ganoderma applanatum* sensu lato, wood rotting fungi, is one of the most common species in warm temperate Japan and yields abundant spores those rarely germinate. In this study, we will explore the production and dispersal of Basidiospore of *G. applanatum* in Japan. We located several *G. applanatum* sl in Kanazawa city, Ishikawa prf. to sample discharged spores and insects visiting between May to October in 2012 and May to November 2013. There two part of this thesis, first part discuss characteristics of *G. applanatum* in producing, dispersal and germination spores. Results are (1) The locations of sporocarp and seasonal changing give significant difference to the amount spores released by sporocarp (2) Seasonal changes give significantly difference to the size and wall thickness of spore which released from each sporocarp (3) The release of spores did not have a significantly difference to spores size, and vice versa (4) All the environmental variables except height of sporocarp above ground in 2012 have influence significantly to release spore (5) Fresh spores have germinated in condition of 25° and 27°C (6) Only spores contain in feces of *Dance picta* were successfully germinated. Second part discussed the abundance of the insects that visit each sporocarp of Ganoderma. Results are (1) In total 963 insects gathering on 13 sporocarps *G. applanatum* were collected (2) Not all sporocarp visited by same insects; there are some species of insects found to be present in some sporocarp, such as family *Scaphidiidae* (Coleoptera), and family *Drosophilidae* (Diptera). (3) *Scaphisoma japonicum* is the most common insects found visiting sporocarp either in Kakuma or town (4) *Mycodrosophila gratiosa* is the species with the highest individual detected consume Ganoderma spores (5) Thirteen out of 20 species of the dominant families of Coleoptera make spores as feeding habits. Meanwhile, all *Mycodrosophila* flies make spores as feeding habits (6) Seasonal change also affects the number of insects that visit on each sporocarp (7) The spores release affect the insect visits on sporocarp (8) The number of insect individual captured from sporocarp did not affect the size of spores.

Key words: Discharge, dispersal and germination, Ganoderma, *Mycodrosophila*, *Scaphidiidae*.

CHAPTER 1
GENERAL INTRODUCTION

1.1. Background of the study

Basidiomycetes (phylum Basidiomycota) include mushrooms, puffballs, and shelf fungi. Many basidiomycetes are decomposers of wood. The life cycle of a basidiomycete includes a long-lived dikaryotic mycelium. In response to environmental stimuli, the mycelium reproduces sexually by producing fruiting bodies called basidiocarps. The numerous basidia in a basidiocarp are sources of sexual spores called basidiospores. Sexual reproduction in Basidiomycota takes place in the fruiting body, in specialized structures called basidia. The basidia are itself formed by plasmogamy between mycelia from two different spores. A young basidium occurs as a terminal dikaryotic cell in the fertile area. Their two haploid nuclei undergo karyogamy (Karyogamy: Process of combining nuclie making ploidity 2n), and immediately the new diploid nucleus undergoes meiosis to form four haploid nuclei. Meanwhile, four, often elongated, projections, the sterigmata, are formed at the basidium apex. The tip of each sterigma balloons out to form the basidiospore initial, and then a nucleus and some cytoplasm migrate into the basidiospore initial from the basidium. Additional wall layers are produced around the basidiospore as it matures. At maturity, most basidia bear four basidiospores.

Characteristics of the genus *Ganoderma*

Ganoderma species discharge characteristic basidiospores which outer thick transparent substances coat the brown-colored inner wall with projections. Nuss (1982) has reported that dimorphic spores present in annual sporocarps of two species, *G. lucidum*, and *G. carnosum*. Spores of these two species changes in its size and its germination physiology, i.e., sporocarps discharge smaller spores those are ready to germinate in the early period of spore-releasing period, whereas, same sporocarps do larger normal spores doubled in transect-space with thicker outer coating those are hard to germinate (Nuss 1982).

Lim（1977）studied effect of arthropod digestion on spore germination of *G. pseudoferreum* using larvae and imago of several arthropod species to found that spores excreted by larvae of Limonia (Diptera: Limnobiidae) germinate (5.9%) while no spores, freshly discharged, excreted by mites, beetles, or drosophyliid flies, did not. Kadowaki (2011) reported that beetles feed on the spores of Ganoderma species mechanically destroy inside walls to degrade the germination ability.

The heart rotting fungi, *Ganoderma applanatum* (Syn. Elfvingia applanata)

One of the most common species in Japan that produce perennial sporocarps. Wood rotting by *G. applanatum* may yield tree cavities which are utilized as nests by a variety of animals, i.e., birds, small mammals, and insects. Therefore, *G. applanatum* can facilitate species diversity of sylvan animal through creating nests for them.

Characteristics of *G. applanatum.*

Physiological feature the spores are under dormancy to germinate irregularly spending about one year, and keeping spores under high temperature (30-40°C) breaks spores dormancy (Aoshima 1954). Spores of *Elfvingia applanata* (= *G. applanatum*) are frequently fed by Mycodrosophila flies (Drosophilidae), Scaphisoma beetles (Scaphidiidae) and other beetle species (Tuno 1999). Mycodrosophila flies digest only the outer coating matter to keep inside walls being undestroyed by SEM observation and trypan blue stain (Tuno 1999). Digested spores in flies feces can germinate (Shimotoku and Tuno 2012 unpubl.data).

1.2. Objectives of study

In forestry, fungi and insects play a crucial role. Their presences are directly or indirectly have an impact on the growth of trees and also on timber logging in natural forests or plantations. Both fungi and insect are one of the most factors to damage tree or wood in place wood storage. Especially for G. *applanatum,* wood-decay fungus is one of the most common species in warm

temperate Japan and yields abundant spores those rarely germinate. *Ganoderma sp*. is one of the fungi species that have mutually interacts with some insects.

This study will explore the production and dispersal of Basidiospore of *G. applanatum.* We will address some questions. ***First***, how does spore production, spore size, its germination rate differ among fungal genet?: genet (A clonal colony) is a group of genetically identical individual, such as plants, fungi, or bacteria, which have grown in a given location, all originating vegetatively, not sexually, from a single ancestor. ***Second***, how to do digestion of spores by animal affect on spore germination?. ***Third***, does insect abundance have influence on spore production, and spore size?.

1.3. Organization of the study

This dissertation is the result of four years study on the production and dissemination of basidiospores of *Ganoderma applanatum* in western Japan. Interactions between Ganoderma and insects visiting the sporocarp also discussed in this paper. The results of this study are organized into four chapters.

The first chapter begins with a brief introduction on the background and objectives of this study.

The second chapter contains the characteristics of *G. applanatum* in releasing spores. Besides, this chapter also discussed the germination of Ganoderma spores and germination of spores that have been feeding by insects (feces that contain spores).

The third chapter discussed the abundance of the insects that visit each sporocarp of Ganoderma. The influence of the presence and not present spores; and spore size to the presence of insects is also discussed in this chapter.

The fourth chapter is the final chapter in this dissertation. This chapter provides a summary of all the results from this study. In this chapter, I also wrote the concluding remarks and the scope for further research.

CHAPTER 2
CHARACTERISTICS OF *Ganoderma applanatum* (GANODERMATACEAE, BASIDIOMYCOTA) IN RELEASING SPORES

2.1. Introduction

Ganoderma applanatum is a perennial fungus with a thick outer-wall of spore. This fungus often found on dead hardwood, but also on a weak or wound parasite, commonly on *Fagus* (beech), *Acer* (maple), *Tilia* (linden), *Fraxinus* (ash), *Populus* (poplar), *Quercus* (oak), *Salix* (willow), more rarely on conifers. It is easy to recognize this fungus in the field. Overall fruit body form, very similar to *G. adspersum* but pore surface features and spore size differs. Especially in USA, this fungus also famous with the folk name ‘’*designer's mushroom*‘’ and ‘’*artist's conk*", it is because we can drawings using a finger nail on the fresh pore surface. The frequently, we also found insect-gall on the pore surface. (Breitenbach and Kränzlin 1986; Buczacki 1989).

When the fungus release spores, they also need an external force to help them release the spores. There is three source of the force that help fungi to release spore; wind, water and animal. The wind is one of provider that help spores releasing from fruit body. Conidia of powdery mildew and many of downy mildew may be blown from their birthplace soon because of the wind. This winding method is a very simple compare than other release methods. Water, as raindrop, can release spores from their anchorage. Raindrops are forcing out the egg on a tiny nest-like fruit bodies that contain Basidiospores. This method is a primary release method for conidia on many Deuteromycetes molds. Ganoderma discharge spore with the extremely refined mechanics of fruits body. Ganoderma has tube may ultimately around 5 cm long but only 0.1 or 0.2 mm in diameter. Spores are driven with such accuracy to the center of this tube and fall vertically downwards them with precision so that very little stuck on the side but the rain fell in a continuous stream at a rate of several million minutes for 24 hours a day and more than six months a year (Buczacki, 1989). Animals (especially insects) also played their part in the release of spores. Because of tiny size of spores, small animals tend to assist spore on their way (Buczacki, 1989). Interaction

between insect and fungus can be antagonistic and/or mutualistic. In natural conditions, insect-fungus interactions take part in many processes of forest ecosystems. For example in the forest, fungus and insects play a predominant role in recycling of litter on the forest floor. Because of its variability and relative durability, wood provides a large number of various niches for a highly diverse fungal and insect community. (Muller et al., 2002). *Ganoderma sp*, one of the fungi species, mutually interacts with some insects. Tuno (1999) found *Mycodrosophila oldenburg* has been used by *G. applanatum* as media for spore dispersal.

After discharge, spore dispersal is assisted by other agencies that play a role in the next process. For some species, this process gives the separation of a parent fungus or colonies for successful growth and adequate nutrition. But for most species of fungi, the spores' further deployment is required, and the process resembled seeds, fruits and pollen-grains of higher plants. (Buczacki, 1989). This spore dispersal process also requires the role of both living and non-living agents. Furthermore, role of the environment is also needed to make the spores becomes stronger to survive. The wind plays a very significant role in the process of spore dispersal; it also supported with a small size and very light weight of spore. The wind carries the spores far and away, and the wind is an agent of the most important carriers of spores. The rain also plays a significant role in the spread of spores, especially for short distance spore dispersal (Buczacki, 1989).

Dispersal of fungal spores by animals also occur in various forms; for instance: eaten vegetation or spores carried on the hair or fur of the animal. Almost all groups of the kingdom of animals from nematodes to mammals have representation and roles involved in the spore dispersal. Insects are one of the animals that have a crucial role in the spore dispersal. Many studies discuss the role of insect in helping to the dispersal of the fungal spores. (Buczacki, 1989). For example, Tuno (1999) found insect visiting sporocarps of *Elfingia applanata*, a wood-rotting bracket fungus, were supposed to migrate between the sporocarps of other bracket fungi growing on different logs or stumps, suggesting that *Mycodrosophila* flies may act as spore-dispersal agents. The most dominating spore feeding insects are species of beetles (Coleoptera) and *Mycodrosophila*

species (Drosophilidae, Diptera) and suggested that flies may contribute to fungal spore dispersal since flies excrement consists of number of intact spores.

At the time spores release from the parent fungus; spores have the opportunity to land in a safe area and then began to germinate. It is the most important process for spores, it must germinate. We can analogies the spore germination process as the process of germination in plants (seeds). As well as seeds, not all spores were able to germinate immediately. There are spores in the dormant condition, so they take a time for few weeks or months and or they need special treatment to make it germinate. (Buczacki, 1989). As one of the basidiospores, *Ganoderma sp.* often remains either dormant spore or, if the spores germinate; it was very slowly or at an extremely low rate (Fries 1966 in Lim 1977). The germination of basidiospores of *G. applanatum* was very erratic and when it did occur, germination took place within 48 hours after the basidiospores had placed on agar media, in water or other liquid media, but the germination percentage thus obtained was tiny, being about 1.5 percent. To break dormancy spores of this fungus is needed exposure to high temperatures which done in dry conditions and the water. This fungus has spores with thick and unique structure of their cell walls. Therefore, a phenomenon of their dormancy, inducing them to break dormancy is unique in this spores. (Aohima 1954)

2.2. Materials and methods

2.2.1. Study sites

We studied sporocarps of *Ganoderma applanatum* (Ganodermataceae) in two study sites of Kanazawa City, Ishikawa Prefecture, western middle of Japan. The first site is in the city center (36°56'N, 136°66'E, Fig. 1) and is called as town hereafter. In the city center, sporocarps were found in scattered living trees of *Cercidiphyllum japonicum* or Prunus species in the parks or the museum. The second site is located in the Kakuma campus of Kanazawa University (36°55'N, 136°71'E, Fig. 1) and is called as Kakuma hereafter. The sporocarps were found in the secondary forest dominated by *Quercus variabilis* and *Quercus serrate* except for K6 (Fig. 1) which is from a stump of *C.*

japonicum by the road. Kakuma is located about 4Km away from town (Fig. 1).

In town, we studied six out of 10 sporocarps found in both of 2012 and 2013. In Kakuma, we did five out of six found in 2012 and four out of four in 2013. We assumed plural sporocarps found in one tree or a decaying wood as one fungal genet and studied only one of them to represent the fungal genet. During the observation, all sporocarp was still alive (Fig. 2). It is characterized by the white to cream color of pore surface; sporocarp still releasing spore (except sporocarp on T4 and T5) and the presence of insect activity on the sporocarp (hymenophore with insect and insect gall) (Breitenbach and Kränzlin 1986). In the end of observation (the end of October until early November 2013) sporocarp on T4 and T5 began to decay.

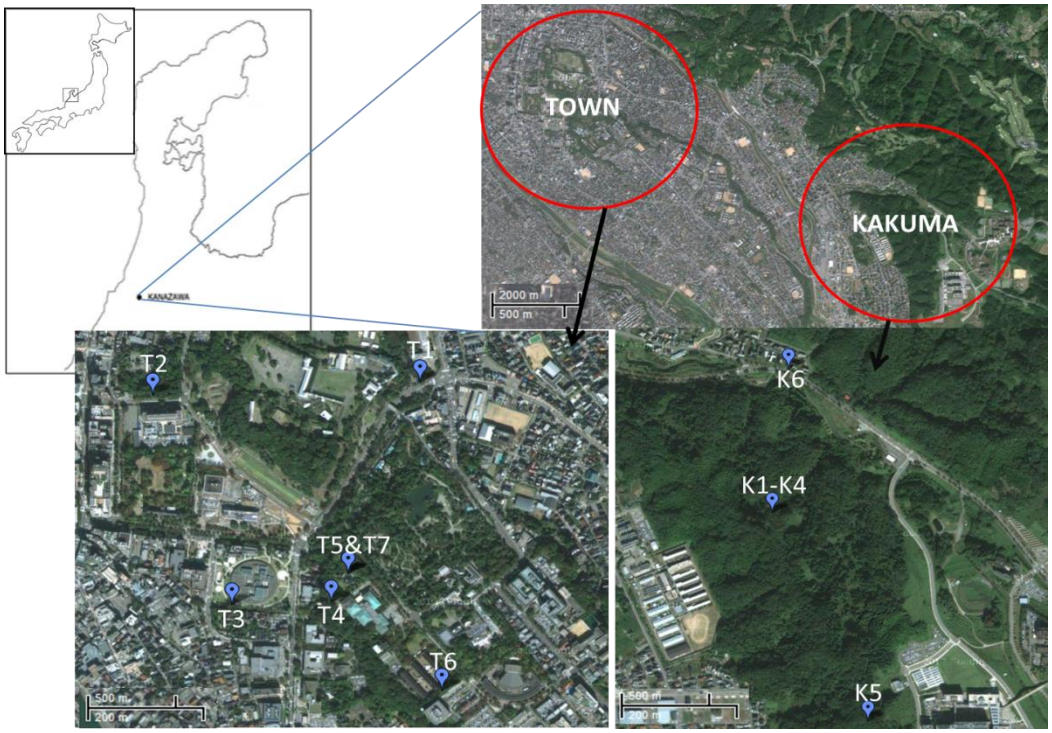

Fig. 1 Map of *Ganoderma applanatum* sensu lato (Ganodermataceae, Basidiomycota) at Kanazawa City, Ishikawa Pref. Japan

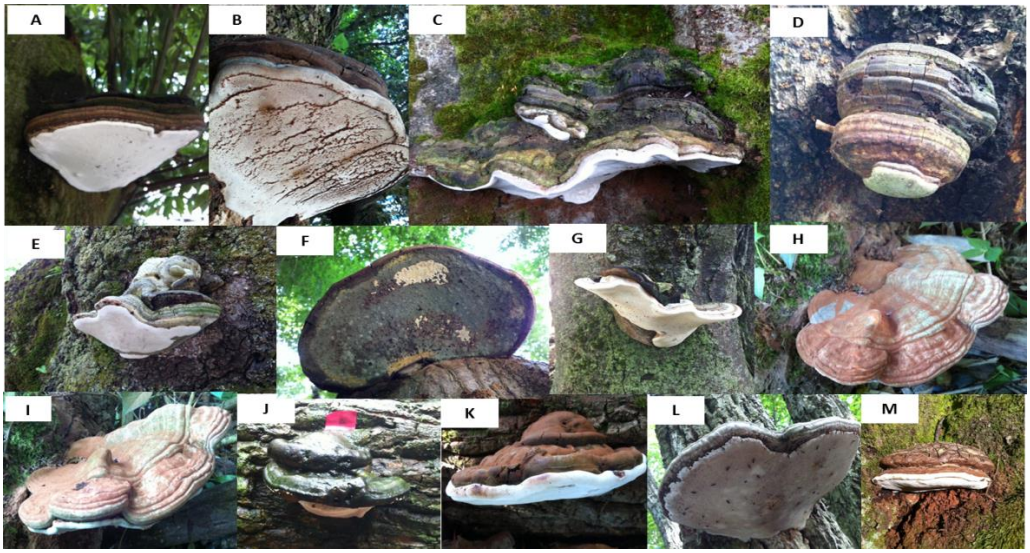

Fig. 2 Ganoderma applanatum at Kanazawa City, Ishikawa Pref. Japan. A. Hokucho-ro Str (Near Maeda Toshiie Statue) (T1); B. Oyama Tample (T2); C. 21st Century Museum of Contrepory Art, Kanazawa (T3); D. Behind Pref. Museum of Art (T4); E. In front of Pref. Mu E. In front of Pref. Museum of Art Hirosaka Annex Section (T5); F. Behind Pref. History Museum (T6); G. In front of Pref. Museum of Art Hirosaka Annex Section I (T7); H. Kakuma Forest (Tower 1) (K1); I. Kakuma Forest (Tower 2) (K2); J. Kakuma Forest (Bamboo area 1) (K3); K. Kakuma Forest (Bamboo area 2) (K4); L. Kakuma Forest (K5); M. Highway 27 Ishikawa, Kakumaguchi (K6)

Table 1 is the list of sporocarps location both in Town and Kakuma Number one until seven are located in Town. Number eight until thirteen are located in Kakuma.

TABLE 1. Location of sporocarps Ganoderma applanatum observed in town (T1-T7) and in Kakuma (K1-K6) at Kanazawa City, Ishikawa Pref. Japan

No	Location	Fig 2. Label	Code
1	Hokucho-ro Str (Near Maeda Toshiie Statue)	A	T1
2	Oyama Tample	B	T2
3	21st Century Museum of Contrepory Art, Kanazawa	C	T3
4	Behind Pref. Museum of Art	D	T4
5	In front of Pref. Museum of Art Hirosaka Annex Section	E	T5
6	Behind Pref. History Museum	F	T6
7	In front of Pref. Museum of Art Hirosaka Annex Section I	G	T7
8	Kakuma Forest (Tower 1)	H	K1
9	Kakuma Forest (Tower 2) (K2);	I	K2
10	Kakuma Forest (Bamboo area 1)	J	K3
11	Kakuma Forest (Bamboo area 2)	K	K4
12	Kakuma Forest	L	K5
13	Highway 27 Ishikawa, Kakumaguchi	M	K6

2.2.2. Spores sampling

The periodicity of spore release from respective sporocarps was quantified for once or twice in a week between May to October or November to cover the whole spore-releasing period. We put a filter paper (diameter 7.0 cm) in 2012 or a plastic plate (3.7 cm x 2.3 cm x 0.2 mm) in 2013 under hymenophores for 24 hours to collect released spores. Discharged spore amounts were expressed as weight in 2012 while as a number of spores in 2013. In 2013, each spore print was suspended in 10 ml of distilled water to measure spore density using a hemocytometer under a microscope. Each spore sample was counted three times and averaged to estimate whole spore number in them.

2.2.3. Measurement of spores

The size of spores was measured under a microscope (x400) with the aid of a digital camera (Nikon Camera Head DS-L3). Thirty spores from respective sporocarps once in a month were measured for width and length for both inner and outer wall and the thickness. The thickness of the outer wall was expressed as a half of the difference between the outer and inner width (Fig. 3). Two kinds of data taken for spore sizes: outer size and inner size. Measurement were represented by cross-sectional areas (S) applying elliptical area calculation ($S=\pi ab/4$, Fig. 3).

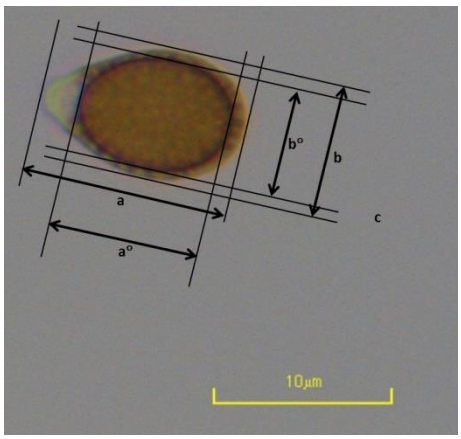

Fig. 3 Parameters of spore measured
(a)(a^o)Lengths of inner and outer wall、 (b)(b^o)Widths of inner and outer wall、(c)thickness as the difference of ½ (b – b^o)

2.2.4. Viability of spores.

We collect feces of insect that contain spore to test the ability of spore germination. Feces of insect put into Eppendorf tube containing one milliliter of water. For controls, make a fresh spore suspension by inserting 20 milligrams of spores derived from sporocarp into an Eppendorf tube containing one milliliter of water. Ten microliters of the homogenized suspension from feces of insect and fresh spore inoculated on PDA media in Petri dish. Three replicated made from each feces suspension, and control. Cultures incubated at 22, 25, 27, and 30°C; and tested for germination rates at 1, 2, 4, 8, 12 days after inoculation. Colonies of the fungus that occurred in the cultures were identified by comparing species-specific colony shapes with those in the control culture. Spore germination rates estimated under the microscope (x100) by directly counting the number of spores germinate in 100 randomly selected spores.

2.2.5. Data analysis

Multiple regression analysis was used to assess the relationship between spore production and environmental variable. i.e., season, temperature, humidity, characteristics of sporocarp (height above ground, size, and location). Statistical analyzes were performed with JMP 5.0 (SAS Institute, Cary, N.C.; 1989–2003).

Canonical correspondence analysis (CCA) were applied to relate the abundance of the animal taxonomical groups with environmental factors, i.e., releasing of spores or not, releasing spore density, releasing spore amount, location, temperature and humidity (Canoco ver. 4.5) (Ter Braak and Smilauer 1998).

2.3. Results

The spore's production released from sporocarp of *Ganoderma applanatum sensu lato*

The released spore density and abundance per sporocarps in 2012 and 2013 is shown in Fig. 4. In 2012 (Fig. 4A. and B.) for the study site in the town, T3 released the highest average amount of spore per cm2 (111 mg/cm^2) and also

the highest average amount of spore per sporocarp (4592 mg/sporocarp). T5 released the lower average amount of spore per cm^2 (36 mg/cm^2) and also the lower average amount of spore per sporocarp (491 mg/sporocarp). The study site in Kakuma, K4 released the highest average amount of spore per cm^2 (250 mg/cm^2), and K5 released the highest average amount of spore per sporocarp (4881 mg/sporocarp). K3 released the lower average amount of spore per cm^2 (33 mg/cm^2) and also the lower average amount of spore per sporocarp (186 mg/sporocarp). Two sporocarps never release spores during the observations were made (T4 and T6), both located in the town. The locations give significant difference to the amount spores released by sporocarp in 2012 (Kruskal-Wallis Tests; per cm^2: $\chi^2 = 167.01$, d.f = 10, P< 0.0001; per sporocarp: $\chi^2 = 171.67$, d.f = 10, P< 0.0001).

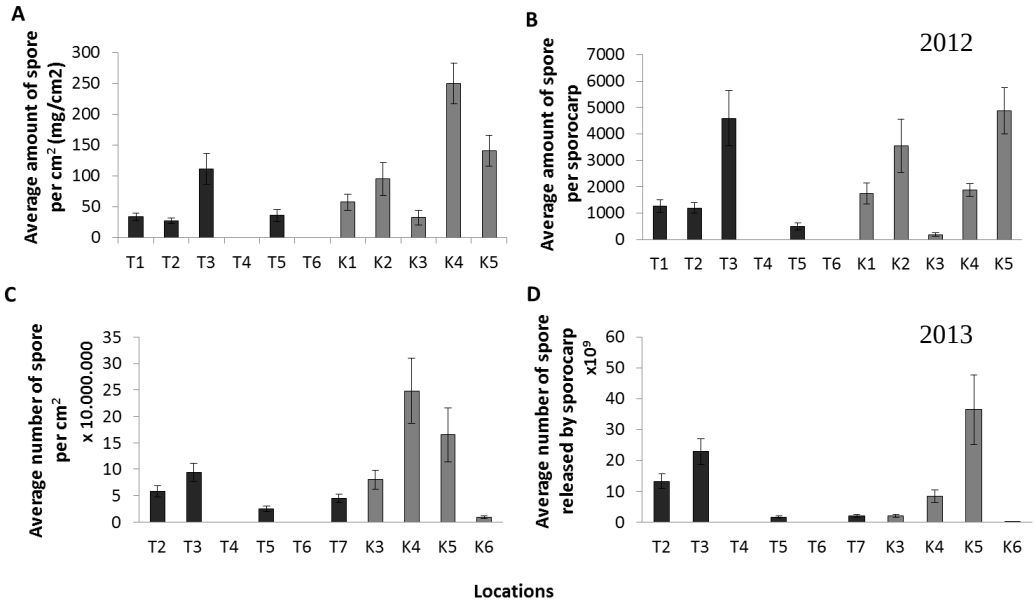

Fig. 4 The released spore density (A and C) and abundance per sporocarps (B and D) in 2012 and 2013.

In 2013 (Fig. 4C and D.) for the study site in the town, T3 released the highest average number of spore per cm^2 ($9.3x10^7/cm^2$) and also the highest average number of spore per sporocarp ($2.3x10^{10}$ /sporocarp). T5 released the lower average number of spore per cm^2 ($2.5x10^7/cm^2$) and also the lower average number of spore per sporocarp ($1.7x10^9$/sporocarp). The study site in Kakuma, K4 released the highest average number of spore per cm^2 ($2.5x10^8/cm^2$), and K5

released the highest average number of spore per sporocarp ($3.7x10^{10}$/sporocarp). K6 released the lower average number of spore per cm^2 ($9x10^6/cm^2$) and also the lower average number of spore per sporocarp ($1.7x10^8$/sporocarp). The same as in 2012, sporocarp at T4 and T6 (both located in the town) never release spores during the observations were made. The locations give significant difference to the number of spore released by sporocarp in 2013 (Kruskal-Wallis Tests; per cm^2: $\chi^2 = 125.35$, d.f = 9, P< 0.0001; per sporocarp: $\chi^2 = 147.89$, d.f = 9, P< 0.0001).

The six out of 11 sporocarps have been starting to release spores in May 2012 (Fig. 5A.); there are T2, T3, K1, K2, K4, and K5. The seasonal changing gives significant difference to spore production released by sporocarp in 2012 (Kruskal-Wallis Tests; per cm^2: $\chi^2 = 54.15$, d.f = 5, P< 0.0001; per sporocarp: $\chi^2 = 50.61$, d.f = 5, P< 0.0001). There are similarities in the pattern of release of spores per plate and sporocarp. There are had been an increasing average amount of spores discharge by sporocarp in May to July (8.84 mg/cm2 and 229.92 mg/sporocarp). The number abruptly decreased in August and started to rise again in September (11.17 mg/cm2 and 287.72 mg/sporocarp). In October 2012, several sporocarps look still discharging spores.

The same as in 2012, six out of ten sporocarps have been starting discharge spores in May 2013 (Fig. 5B.), there are T2, T3, T5, K3, K4, and K5. In 2013, seasonal changing also gives significant difference to average number of spore released by sporocarp (Kruskal-Wallis Tests; per cm^2: $\chi^2 = 50.38$, d.f = 6, P< 0.0001; per sporocarp: $\chi^2 = 44.19$, d.f = 6, P< 0.0001). The average number of spore per cm^2 increased from May to July ($1.3x10^7$ spores/cm^2) and after that decreased until the end of the observation. The average number of spore per sporocarp was fluctuating. In November, several sporocarp looks still releasing spores.

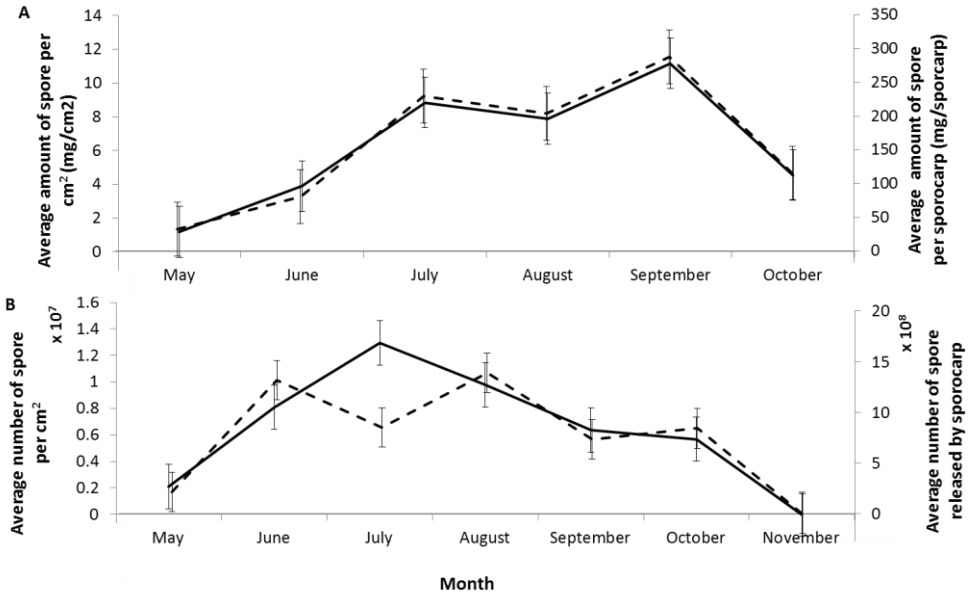

Fig. 5 Monthly changes of released spore abundance from all sporocarps in 2012 (A) and 2013 (B). *Solid line* represents average amount/number of spore per cm^2 and *dashed line* represents average amount/number of spore per sporocarp.

Change of spore size and wall thickness among sporocarp *Ganoderma applanatum*

Sporocarp discharged spores in a different size (outer and inner) and wall thickness. At the time of a sporocarps release spores, sporocarps will release spores in various sizes and wall thickness. However, this does not apply to all sporocarps. Several sporocarps release spores with sizes and wall thickness that do not differ significantly (Fig. 6).

Outer size ranged between 215.87 – 293.32µm2 in 2012 and 205.61-294.33µm2 in 2013. The largest size released by sporocarp in T3 (2012 and 2013) at the town. The smallest size released by sporocarp in T1 (2012) at the town and K3 (2013) at Kakuma. All sporocarps except in K3 have significant difference in outer sizes in 2012, whereas, in 2013, all sporocarps except in K6 have significant difference in outer sizes

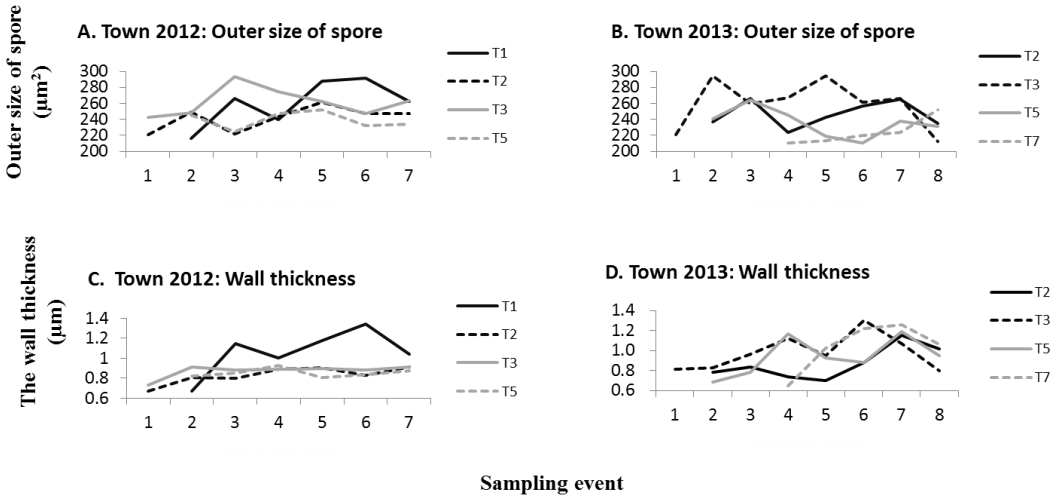

Fig. 6 The size and wall thickness of spore released from sporocarp *Ganoderma applanatum* (Ganodermataceae, Basidiomycota).

The wall thickness of spores ranged from 0.67 -1.34µm (2012) and 0.54-1.39µm (2013). Wall thickest released in T1 at the town (2012) and K5 at kakuma (2013). While wall thinnest released in T2 at the town (2012) and K4 at Kakuma (2013). In 2012, the wall thickness of spore from sporocarp in T1, T2, T5, K1, K2, and K5 were significantly different. In 2013, all sporocarps except in T7 and K6 had significant difference in wall thickness.

The relationships between spore size and spore abundance at sporocarp of *Ganoderma applanatum*

The relationships between spore size and spore abundance per sporocarp shown in Fig. 7. There is no relationship between spore abundance and spore size (2012: ANOVA, F=1.74, P=0.19; 2013: ANOVA, F=1.66, P=0.2). When sporocarp was released spores in large numbers, spores size are not always large or small.

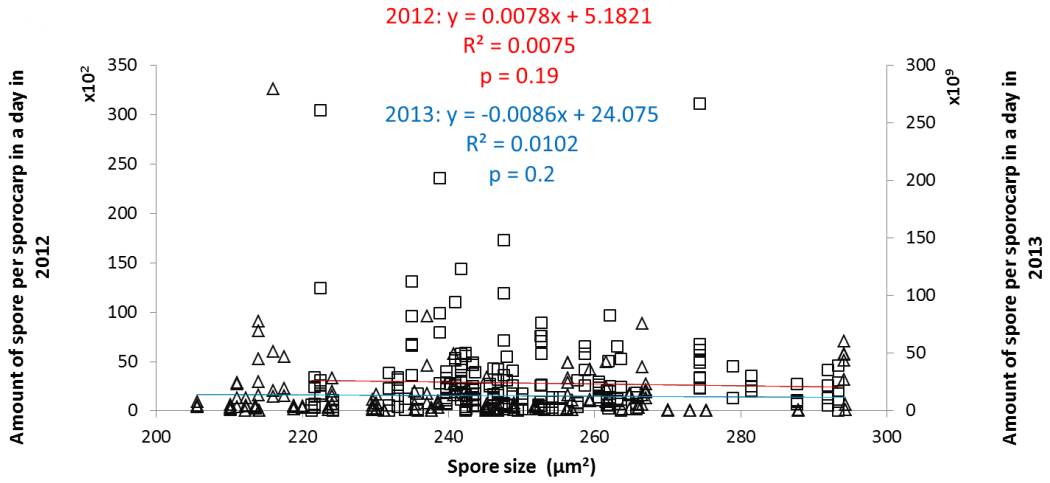

Fig. 7 The relationships between spore size and spore abundance released sporocarp of *Ganoderma applanatum* (Ganodermataceae, Basidiomycota). *Square and red line* represents sample event in 2012; *triangle and blue line* represents sample event in 2013.

Influence of environmental variables on spore abundance at the sporocarp of *Ganoderma applanatum*

The influence of the environmental variable on the release of spore abundance (temperature and relative humidity) shown in Fig. 8. Temperature and relative humidity (except humidity in 2012) showed the positive correlations with the release of spore abundance in general linear model analyses (Fig. 8). The temperature had influence significantly to release of spore abundance in 2012 and 2013 (2012: ANOVA, F=12.57, P<.001; 2013: ANOVA, F=17.36, P<.0001; Fig. 8A). Relative humidity has influence significantly to release of spore abundance in 2013 (ANOVA, F=4.86, P<.05; Fig. 8B).

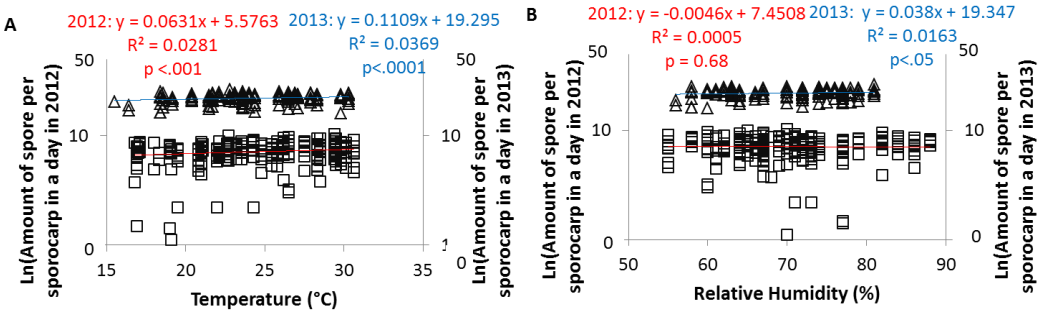

Fig. 8 The influence of environmental variables on spore abundance: A. Temperature (°C); B. Relative humidity (%). *Square and red line* represents sample event in 2012; *triangle and blue line* represents sample event in 2013.

The influence of height of sporocarp above ground and sporocarp size on the release of spore density shown in Fig. 9. The height of sporocarp above ground showed negative correlations with the release of spore density in general linear model analyses, but it has influence significantly to release of spores density in 2012 and 2013 (2012: ANOVA, F=23.92, P<.0001; 2013: ANOVA, F=26.63, P<.0001; Fig. 9A).

Sporocarp size showed the positive correlations with spore release in general linear model analyses in 2013. Sporocarp size has influence significantly to spores release in 2012 and 2013 (2012: ANOVA, F=45.67, P<.0001; 2013: ANOVA, F=139.04, P<.0001; Fig. 9B).

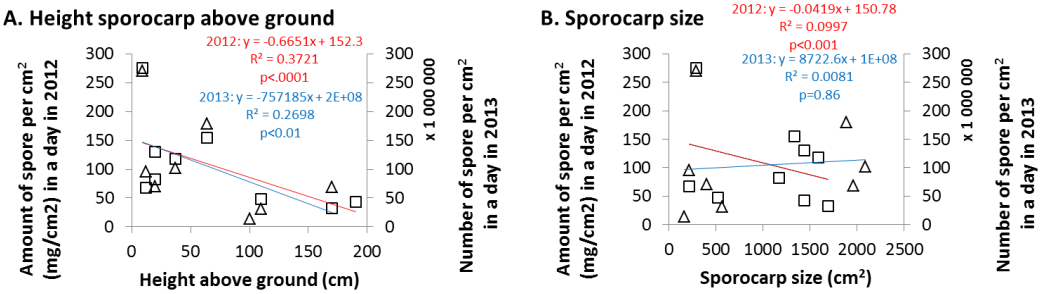

Fig. 9 The influence of height of sporocarp above ground and sporocarp size on release of spore density: A. Height sporocarp above ground (cm); B. Sporocarp size (cm^2). *Square and red line* represents sample event in 2012; *triangle and blue line* represents sample event in 2013.

Germination rate of spore *Ganoderma applanatum*

Spores were first released by sporocarp be used as the material for spore germination test. Twenty-four hours later after spore collection, spores cultivated on PDA and simple agar. Spores were taken from four locations: T2, K3, K4 and K5. Spore germination of ganoderma at the different temperature shown in Fig. 10A. The spores have germinated in conditions of 25° and 27°C. FigurFig. 10B shows spore germination day after inoculation at 25°C using PDA. At 25°C, the spores begin to germinate on the second day after inoculation, except spores of K3, which started to germinate on the eighth day after inoculation (Fig. 10B). Spore did not germinate at any in agar without nutrients.

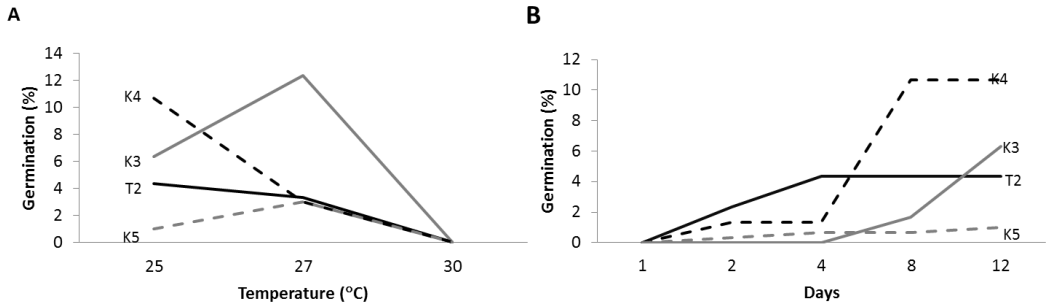

Fig. 10 Spore germination rate (%) at different temperature (A) and changes in days after inoculation at 25°C (B). *Black solid line* represents germination spore from T2; *Gray solid line* represents germination spore from K3; *Black dashed line* represents germination spore from K4; *Gray dashed line* represents germination spore from K5.

Insects have a role in spores dispersal. One method of spore dispersal by insects is by consuming the spores and removes it in the form of feces. There are times when spores contained in the insect feces are still in good condition (intact), but some are in broken condition. Germination spores test are done at insect feces. We used some beetle that was *Cyllodes dubious, Dance picta, Xylographus scheerpeltzi, Aphanocephalus hemisphericus, Scaphisoma haemorrhoidale* and *Biphyllus humeralis* for collection their feces. Among six beetles were tested, only spores contain in feces of *D. picta* started to germinate. These spores germinate on the second day after inoculation under conditions of 25°C (Fig. 11).

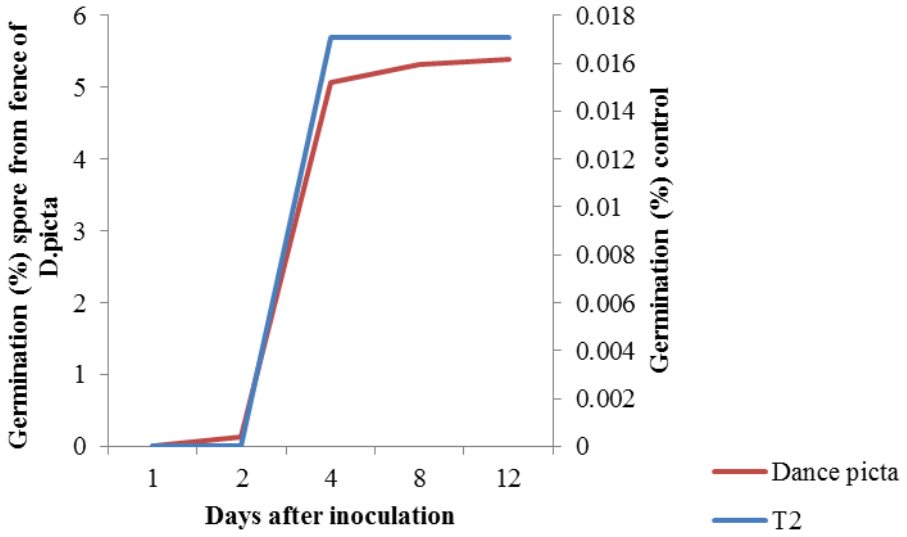

Fig. 11 Germination of *Ganoderma applanatum* (Ganodermataceae, Basidiomycota) spore from feces of *Dance picta* at 25°C.

2.5. Discussion

Sporocarps Ganoderma is unique; annually sporocarps will form a new hymenium layer. If sporocarp is no longer form a hymenium, meaning sporocarps is already getting old and over time will rot and die. If sporocarps still form the hymenium layer, meaning sporocarps still will produce spores.

Spore production differs between sporocarps. The release of spores is determined by factors that are at from sporocarp itself (for example age of sporocarp) and factors that come from outside sporocarp (environment factor). Sporocarp location is a factor that influences to the success of sporocarp to releasing spores. Meanwhile, the changes of seasons also determine the amount of spore production by sporocarp.

The influence of environmental factors is one of cause sporocarp release spores with different amounts. At one sporocarp, the first observations were made (May) until the end of the observation (October/November) will appear change of spore production. Initial observations were conducted in May showed that only a few sporocarp was already releasing spores. The whole sporocarps begin releasing spores observed in June and the highest release in July. Sporocarps stop releasing spores in late October. According to Kadowaki (2010) release spores of the

wood-rotting basidiomycete, Ganoderma is a very fluctuating pattern and weak seasonal. Among sporocarps observed, two sporocarps do not produce spores throughout the observation lasted. The second year of observation, both sporocarps began to show signs of decay.

Four components of the environment are measured to see the connection with the spore production. There is temperature, humidity, height of sporocarp above ground and sporocarp size. Temperature and humidity in 2013 showed a significant positive relationship with spore abundance. Sporocarp size, of course, is one of the determinants of the number of spores produced by a sporocarp, but the number of spores per plate was the most certain amount of spore production. If two of sporocarp has nearly the same size, then sporocarp that produces spores per plates most higher the spore-producing most.

Sporocarp will release spores with a different size. Spore size and abundance differed among sporocarps and changed in the course of time. The seasonal change also gives influence to spore size. However, there is no relationship between spore size and released abundance. At the time of sporocarps release spores in large quantities, the size of the spores was released on that occasion can be large or small.

CHAPTER 3
INSECT ASSEMBLY GATHERING ON SPOROCARPS OF *GANODERMA APPLANATUM* (GANODERMATACEAE, BASIDIOMYCOTA)

3.1. Introduction

Sporocarps of fungi provide habitat for the wealth of non-insect micro-arthropods and abundance of insect. Acari (mites) and Collembola (springtails) are the main group microarthropods associated with sporocarp while Diptera (flies) and Coleoptera (beetles) dominate the insect community. Together these arthropods both obligate and facultative consists of sporocarps users and their communities encompass the diversity of nutritional modes. Although many are mycophagous and active grazing fungal spores and mycelia, others are, predators or parasitoids are looking prey or hosts on sporocarps. (Epps and Arnold 2010). Many basidiomycetes have various kinds of relationships with insects. For example, the symbiosis between insects and wood-decaying basidiomycetes involves woodwasps (Hymenoptera; Siricidae) (Gilbertson 1984).

Insects use fungi as food or as a breeding site does not necessarily destroy the host spores. When a spore formation is not disturbed, and not all spores are consumed, there is a possibility that the insects will disperse host spores. This is often right ingested spores are not damaged in the digestive tract of insects. Feeding at basidiomes may facultative or obligatory for fungivorous beetles. Insects, especially Diptera and Coleoptera, are the animal most often utilize the resources of the fungus. (Blackwell 1984; Graf-Peters et al. 2011)

Fungivorous insects are viewed as polyphagous, especially since most fungal fruiting bodies are the unexpected source. Polyphagy of fungivorous insect is mainly indicated for the insects associated with fungi. Insects fungivorous are regarded as specific in selecting a host. Insects that depend on fungi as food and shelter at all stages of their development called mycetobionts. (Jonsell and Nordlander 2004; Graf-Peters et al. 2011).

3.2. Materials and methods

3.2.1. Study sites

Areas of study in this chapter same with area sites in the previous chapter (chapter two). Insects collected from sporocarps at the same location (Fig. 1).

3.2.2. Insects sampling

All adult insects visiting the sporocarps were collected by an aspirator with the aid of a hand net, weekly from May to October in 2012 and May to November in 2013. Insect collections made between 8 am to 5 pm. The sampling order was shifted to avoid biases of the sampling hours from them. Studied sporocarps were evenly sampled at different hours in every month. We observed behavior and feeding habit of insects for 15 minutes prior to the collection. The collected insects were kept alive in glass vials to bring them laboratory for further investigation. Collected insects were identified their taxa, i.e., order, family, genus, or species, referring to Uéno et al. (1985) and Kurosawa et al. (1985) for Coleoptera, Okada (1956 and 1968a,b.) for Drosophilidae, and Borror et al. (1992) for other insects.

3.2.3. Feed Habit

Collected insects those visited the sporocarps were confirmed their feeding habits by the following process in the laboratory. First, collected insects were placed in a petri dish individually, supplied with a piece of cotton containing water for 24 h at 25°C, to collect and examine their feces. Second, the insect was dissected to examine the content of their digestive tract under the microscope. We recorded if spores of *G. applanatum* in their digestive tract.

3.2.4. Data analysis

We applied canonical correspondence analysis (CCA) to relate the abundance of the animal taxonomical groups with environmental factors, i.e., location, releasing of spores or not, releasing spore density, releasing spore amount, temperature, and humidity using CANOCO software version 4.5

(Microcomputer Power, Ithaca, NY, USA). This was conducted to identify significant environmental factors determining the animal assemblages in the fields. We determined the significance of relationships between animal density and environmental variables using Monte Carlo permutation tests (n = 500). Firstly we checked if the animal assembly is significantly different between the two locations of town and Kakuma. If it was the case, we repeated CCA applying location of sporocarp (Town and Kakuma) as a covariable. Multiple regression analysis was used to assess the relationship between insect abundance and environmental variable. The environmental factors are spore present or not present; the abundance of the spore, the density of spore, temperature, humidity, seasonal change, and location of the sporocarp (Town and Kakuma). Statistical analyzes were performed with JMP 5.0 (SAS Institute, Cary, N.C.; 1989–2003). The other analyzes were performed using CANOCO version 4 (Ter Braak and Smilauer 1998), and the number of permutations was 499.

3.3. Results

In total 963 (Kakuma: 362 in 2012 and 200 in 2013, Town: 135 in 2012 and 266 in 2013) insects gathering on 13 sporocarps *G. applanatum* were collected in two years. Seven orders of insect found in 2012 and five orders found in 2013. Beetles and genus of *Mycdrosophila* were dominant in number. The most insects were visiting K5 in Kakuma (in total 390 insects in 2012 and 2013) and T3 at town (in total 190 in 2012 and 2013)

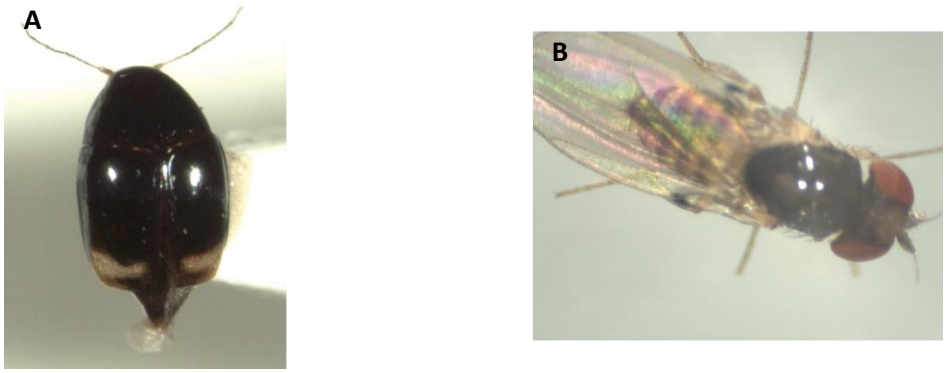

Fig. 12 A: *Scaphisoma sp.* B: *Mycodrosophila sp.*

TABLE 2 show abundance, frequency and feeding habit of species of dominating families in number captured at *G. applanatum*. Coleoptera and Diptera are dominating regarding the number of individuals which visiting sporocarp at each observation site. Most of them spore feeding. Coleoptera and Diptera are always seen present at almost every sporocarp. Species from Coleoptera is the most number of individual found visiting sporocarp of Ganoderma, mainly in the Kakuma. However, Coleopteran is commonly found in 2012 than in 2013. The 631 individuals of Coleoptera found visiting sporocarps. The eight families of Coleoptera are frequently found to visit sporocarps. They are Byrrhidae, Ciidae, Discolomidae, Erotylidae, Mycetophagidae, Nitidulidae, Scaphidiidae, and Tenebrionidae. Scaphidiidae is the most commonly family found to visit sporocarps. *Scaphisoma japonicum* is the dominant species. It also is the most dominant species from all species captured from sporocarps. Seven species of Mycodrosophila found visiting sporocarp. There are *Mycodrosophila erecta*, *M. gratiosa*, *M. japonica*, *M. palmate*, *M. poecilogastra*, *M. shikokuana* and *Mycodrosophila sp*. *M. gratiosa* was the most common species found to visit sporocarp. *S. japonicum* and *M. gratiosa* were the most dominant species captured from sporocarp of Ganoderma.

Fourteen out of 20 species of the dominant families of Coleoptera make spores as feeding habits. Meanwhile, all Mycodrosophila flies make spores as feeding habits. One to 19.38 percent of the presence spores (100 spores counted) found in feces of Coleoptera (dominant family) is a broken condition. Meanwhile for Mycodrosophila flies, 0.22 to 4.15 percent of spores (100 spores counted) found in feces is a broken condition. Observation in 2015, we found that in *C. dubius* feces was contained spore of Ganoderma.

TABLE 2. Abundance, frequency and feeding habit of species of dominating families in number captured at *Ganoderma applanatum* in Kanazawa City, Ishikawa-Japan. Frequency shows the ratio of number of locations where the species caught out of the total number of locations in 2012 and 2013

Order	Family	Species	2012		2013		Feeding Habit	In feces	
			N	Frequency	N	Frequency		%broken spores (100 spore)	Number of dissected insect
Coleoptera	Byrrhidae	*Simplocaria bicolor*	11	0.36	1	0.1	Spore	7.11	3
	Ciidae	*Nipponocis magnus*	2	0.09	0	0	not identified		
		Xylographus scheerpeltzi	33	0.82	28	0.5	Spore	9.33	5
	Discolomidae	*Aphanocephalus hemisphericus*	15	0.27	49	0.4	Spore	4.65	18
	Erotylidae	*Dacne funorum*	0	0	1	0.1	not identified		
		Dacne picta	2	0.18	60	0.3	Spore	4.30	10
		Magalodacne bellula	7	0.18	1	0.1	Spore	2.93	5
	Mycetophagidae	*Mycetophagus grandis*	1	0.09	1	0.1	Spore	2.83	2
		Mycetophagus pustulosus	7	0.18	1	0.1	Spore	5.67	3
	Nitidulidae	*Cyllodes binotatus*	1	0.09	0	0	Spore	1.00	1
		Cyllodes dubius	2	0.18	0	0	Spore*)	7.33	1
		Meligethes nitidicollis	20	0.55	0	0	Spore	1.33	3
	Scaphidiidae	*Ascaphium sulcipenne*	2	0.09	0	0	not identified		
		Scaphisoma austerum	0	0	1	0.1	not identified		
		Scaphisoma haemorrhoidale	20	0.36	30	0.7	Spore	19.38	9
		Scaphisoma japonicum	188	0.55	96	0.7	Spore	10.39	47
	Tenebrionidae	*Ceropria laticollis*	3	0.27	0	0	not identified		
		Platydema nigroaeneum	0	0	1	0.1	not identified		
		Plesiophthalmus nigrocyaneus aeneus	0	0	3	0.2	Spore	17.67	1
		Platydema subfascia	6	0.27	6	0.3	Spore	8.53	5
Diptera	Drosophilidae	*Mycodrosophila erecta*	16	0.18	2	0.1	Spore	2.44	9
		Mycodrosophila gratiosa	22	0.27	102	0.5	Spore	3.65	87
		Mycodrosophila japonica	8	0.27	8	0.2	Spore	4.15	13
		Mycodrosophila palmata	8	0.27	1	0.1	Spore	0.93	9
		Mycodrosophila poecilogastra	2	0.18	2	0.1	Spore	0.22	3
		Mycodrosophila shikokuana	69	0.45	5	0.2	Spore	3.51	34
		Mycodrosophila sp.	13	0.45	0	0	Spore	1.17	4

*) Cyllodes dubius found in 2015

For *Scaphisoma* beetles and *Mycodrosophila* flies, seasonal change causes the difference in the number of visits of insects on sporocarp in 2012 (Kruskal-Wallis Tests, df = 5, χ^2 = 32.99, P< 0.0001) and in 2013 (Kruskal-Wallis Tests, df = 6, χ^2 = 35.41, P< 0.0001; Fig. 13). In the town, the number of *Scaphisoma* beetles visiting sporocarps was same in July until August 2012 while in June 2013 was the highest visit from *Scaphisoma* beetles. In Kakuma, July 2012 was the highest visit from *Scaphisoma* beetles while, in 2013, *Scaphisoma* beetles visiting sporocarps was decreasing on May to August and start to rise again in September. The number of *Mycodrosophila* flies visiting sporocarps at the town was falling in June and starting to increase from July to August. May was the highest visit from *Mycodrosophila* flies in Kakuma but after that, it was starting to decrease by the end of the observation. In 2013, September was the highest visit from *Mycodrosophila* flies at Town while June was the highest visit from *Mycodrosophila* flies at Kakuma.

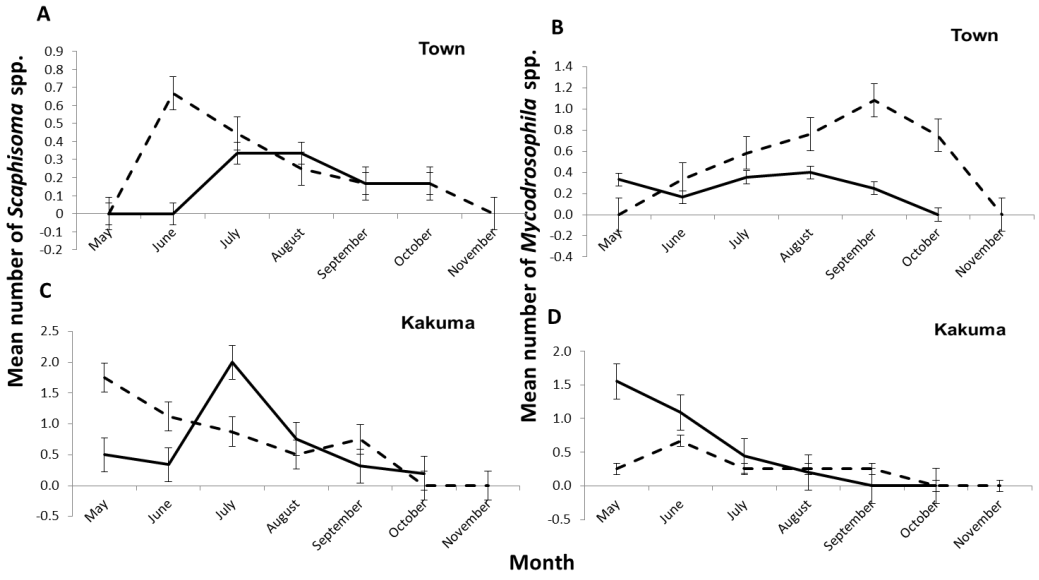

Fig. 13 Seasonal change in average number (per sampling event) of *Scaphisoma* beetles and *Mycodrosophila* flies in 2012 (Solid line) and in 2013 (dashed line): (A, B) in town,(C, D) in Kakuma.

According to CCA analysis results, several environmental factors influence the composition of the insect. Two type environmental factors are using, the first environmental factors are spore present or not present, the abundance of

the spore, the density of spore, temperature, and humidity; and location of sporocarp (Town and Kakuma) as a covariable. The second environmental factors are spore size, the abundance of the spore, the density of spore, temperature, and humidity; location of sporocarp (Town and Kakuma) and spore present as a covariable. The first environmental factors such as the presence or absence of spores, the abundance of spores, spore density and temperature provide a significant influence on the composition of insect species in 2012. Whereas in 2013, the presence or absence of spores, and the abundance of spores are two environmental factors that have a significant influence on the composition of insect species (Table 3A). The second environmental factors such the abundance of spores, spore density, and temperature provide a significant influence on the composition of insect species in 2012. Whereas in 2013, the spores size, abundance of spores and spore density are environmental factors that have a significant influence on the composition of insect species (Table 3B).

Multiple regression analysis results that several environmental factors influence to the abundance of insect. In 2012, environmental factors such as the presence and absence of spores, spore density, the abundance of spores, seasonal change and location (town and Kakuma) had a significant influence on the abundance of insects. The abundance of spores, humidity, seasonal change and location (town and Kakuma) had a significant influence on the abundance of Scaphisoma beetles. The presence and absence of spores, spore density, seasonal change and location (town and Kakuma) had a significant influence on the abundance of Mycodrosophila flies. In 2013, environmental factors such as the presence and absence of spores, the abundance of spores, and seasonal change had a significant influence on the abundance of insects. Spore density, the abundance of spores, seasonal change and location (town and Kakuma) had a significant influence on the abundance of Scaphisoma beetles. The presence and absence of spores, the abundance of spores, and location (town and Kakuma) had a significant influence on the abundance of Mycodrosophila flies. (Table 4)

TABLE 3 The influence of environmental variables on insect's species composition (Summary of CCA analysis)

A	2012		2013	
Monte Carlo Test of significance of first canonical axis				
eigenvalue	0.498		0.458	
F-ratio	6.416		5.844	
P-value	0.002		0.002	
Environment variable	**F**	**P**	**F**	**P**
Spore present	2.99	0.004	1.71	0.036
Ln (Abundance of spore)	1.72	0.036	5.64	0.002
Ln (Density of spore)	4.35	0.002		-
Temperature	5.25	0.002		-
RH		-		-

B	**2012**		**2013**	
Monte Carlo Test of significance of first canonical axis				
eigenvalue	0.437		0.456	
F-ratio	5.711		5.848	
P-value	0.002		0.002	
Environment variable	**F**	**P**	**F**	**P**
Spore size	-	-	4.24	0.002
Ln (Abundance of spore)	1.72	0.02	2.55	0.004
Ln (Density of spore)	4.35	0.002	5.48	0.002
Temperature	4.68	0.002	-	-
RH	-	-	-	-

TABLE 4 Relationships between insect abundance and environmental factors.

Source	2012			2013		
	All insect	Scaphisoma	Mycodrosophila	All insect	Scaphisoma	Mycodrosophila
	RSq 0.14	RSq 0.1	RSq 0.06	RSq 0.27	RSq 0.25	RSq 0.13
	RMSE 3.4829	RMSE 2.6588	RMSE 1.7358	RMSE 3.2454	RMSE 1.515	RMSE 1.8385
	P <.0001	P <.0001	P = 0.0234	P <.0001	P <.0001	P = 0.0008
	P Value					
Spore present	0.0075	ns	0.0193	<.0001	ns	0.0103
Density of spore	0.0334	ns	0.0531	ns	0.0073	ns
Abundance of spore	0.0035	0.0027	ns	0.0035	<.0001	0.0226
Temperature	ns	ns	ns	ns	ns	ns
RH	ns	0.0251	ns	ns	ns	ns
Seasonal (Month)	0.2107	0.6028	0.3246	<.0001	0.0004	ns
Location (Town and Kakuma)	0.0004	0.006	0.0211	ns	0.0134	0.0027

The discharge spores by sporocarp influenced by several factors, such as environmental factors (temperature and humidity). Temperature and humidity have an impact on the continuously of sporocarp in discharging spores. In 2012, the study site had a drier climate conditions compared to the year 2013. During observation times in 2013, sporocarp was continuously releasing spores (Fig. 14C and D). Whereas in 2012, at several locations (especially in Kakuma) seen the release of spores by sporocarp which do not continuously (Fig. 14A and B), when sporocarp do not release spore, some yellow or brown liquid outcome from sporocarp. The insects will come to visit the sporocarp when sporocarp started discharge spores. However, there is still insect found to be present on the sporocarp when sporocarp is not currently releasing spores.

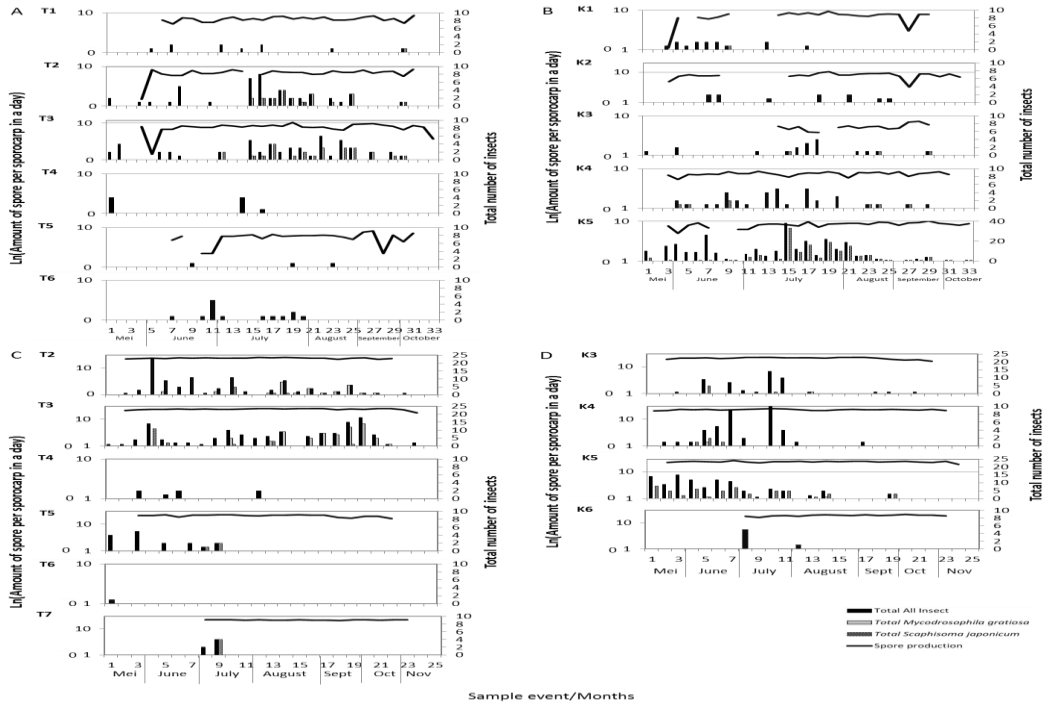

Fig. 14 Spore discharging periode (line) and insect catch (bar) are showhn for respective sporocarps in 2012 (A: Town, B: Kakuma) and 2013 (C: Town, D: Kakuma)

When we did a dissection of the digestive tract of insects done, we not only found spores of Ganoderma. In some insects, we also found spores that come from other fungi. The shape and color of the spore are not the same as the spores are collected directly from sporocarp of Ganoderma. These findings indicate that the insects are not only visiting sporocarp of Ganoderma, but they also visit other fungi.

The presence and absence of spores affect the insect visits on the sporocarp (ANOVA, 2012: F=11.53, P<.001; 2013: F=28.85, P<.001; Fig. 13. and 14). The discharges of spores by sporocarp give no effect on the number of insects which visit the sporocarp. Visiting *Scaphisoma* beetles to sporocarp was not affected by the discharge of spores by sporocarp (2012: ANOVA, F=0.62, P=0.44; 2013: ANOVA, F=3.25, P=0.08). However, especially in *S. japonicum*, visiting this beetle to sporocarp was affected by the discharge of spores by sporocarp (2012: ANOVA, F=17.29, P<.0001; 2013: ANOVA, F=31.75, P<.0001). As with *Scaphisoma* beetles, visiting *Mycodrosophila* flies to sporocarp also not affected by the discharge of spores by sporocarp (2012: ANOVA, F=0.23, P=0.64; 2013: ANOVA, F=0.34, P=0.57). Visiting *M. gratiosa* to sporocarp was not affected by the discharge of spores by sporocarp in 2012 but in 2013 visiting this flies was affected by the discharge of spores by sporocarp (2012: ANOVA, F=2.39, P=0.12; 2013: ANOVA, F=7.59, P=0.0063). Table 5 show that spore discharge period or not discharge period give the difference to insect abundant in Kakuma (2012) and Town (2012 and 2013). Wilcoxon test results show in Kakuma (2012) P<.001, and in Town P=0.0139 (2012) and P<.0001 (2013).

TABLE 5 Insect abundance during spore discharge period and not discharge period

Year	Location	Spore discharging				Wilcoxon test* *P* value
		Yes		No		
		N of insect	No of sampling	N of insect	No of sampling	
2012	Kakuma	345	123	17	42	<.001
	Town	99	110	36	88	0.0139
2013	Kakuma	186	83	14	17	**
	Town	252	80	14	70	<.0001

* The tes was applied for data except for October and November when the number of insect were very low
** The number of data for one group (not dischange spore) was too small to compare

The number of insect individual captured from sporocarps was not significant with the size of the spores (2012: ANOVA, F=1.47, P=0.23; 2013: ANOVA, F=0.29, P=0.59; Fig. 15A). Insects will visit sporocarp when

sporocarp discharges spore with smaller or bigger size. Visiting Scaphisoma beetles to sporocarp was not significant with the size spores (2012: ANOVA, F=1.28, P=0.18; 2013: ANOVA, F=2.15, P=0.15; Fig. 15B). As with Scaphisoma beetles, visiting Mycodrosophila flies to sporocarp also not significant with the size spores in 2012 but visiting Mycodrosophila flies to sporocarp had a positive relationship with the size spores in 2013, (2012: ANOVA, F=0.03, P=0.87; 2013: ANOVA, F=11.33, P=0.001; Fig. 15C).

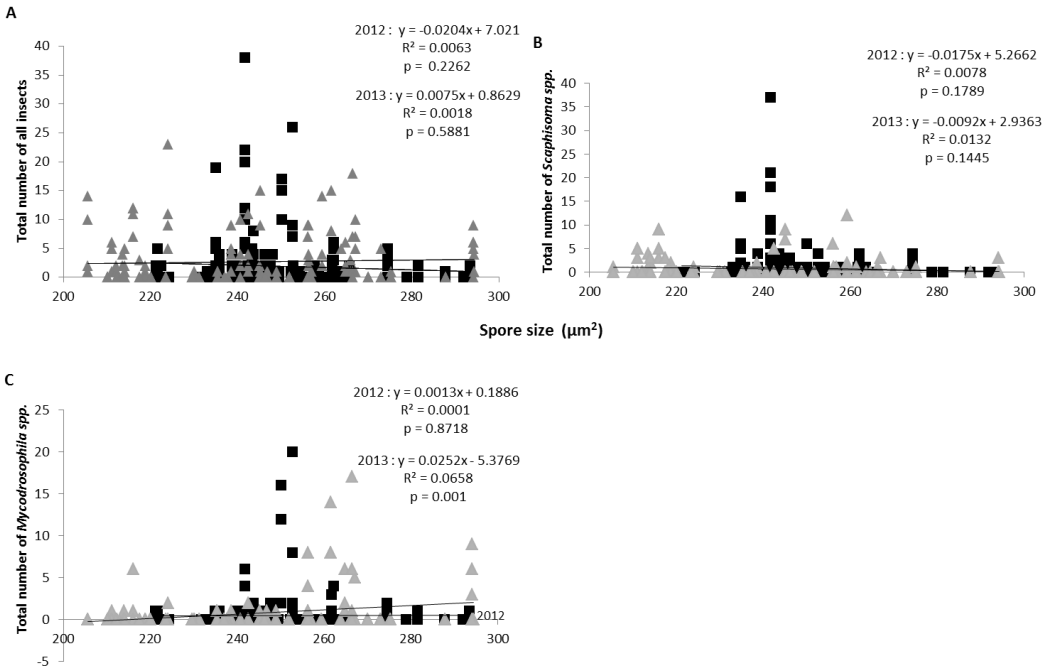

Fig. 15 The relationships between spore size with the number of *Scaphisoma spp*. and *Mycodrosophila spp* individuals captured from sporocarp of *G. applanatum*. *Black square* represents sample event in 2012 and *gray triangle* represents sample event in 2013.

3.4. Discussion

There are several reasons why insects are found in sporocarp of Ganoderma. First, these insects were visiting in sporocarp to feeding spores, which produce by Ganoderma. Second, the insects are not feeding spores but just visiting. Third, these insects are predators, the aim of insect to visiting sporocarp is prey other insects in the sporocarp. Finally, the insects make sporocarp as a host. Not all sporocarp visited by same insects; there are some species of insects found

to be present in some sporocarp, such as family *Scaphidiidae* (Coleoptera), and family *Drosophilidae* (Diptera). Several families of beetles and *Mycodrosophila* flies visited sporocarps to feed spores.

Several orders of insects recorded present at the time when observations were made. Coleoptera and Diptera are the two most dominant orders present in the observations. Although the number of individuals presents from the two orders is not always the same every year. Several families of Coleoptera visit sporocarp to feeding Ganoderma spores. While all *Mycodrosophila* flies certainly were for feeding Ganoderma spores. Tuno (1999) reported *Mycodrosophila* flies were predominant in insect assemblages gathering at sporocarp of *Elfvingia applanata* and visited the spore production sporocarp almost exclusively. *Scaphisoma* beetles came to mature sporocarp of *E. applanata* and ate its spores.

We found eight families of Coleoptera and one family of Diptera, which is the most dominant family present on sporocarp over the observation in 2012 and 2013. However, the frequency of their presence is not same between 2012 and 2013. There are several families present in 2012 and not in 2013, or vice versa. Besides, there is also the presence decreased or increased between those two years. The decrease or increase number of insect may be due to environmental conditions (such as temperature, humidity, seasonal change, the location of sporocarp). If environmental conditions during the observation do not support, it will influence the presence of insects.

As already described previously, not all insects are visiting or present in sporocarp aimed to eat spores. It is also shown on the ruling insect family that presents at sporocarp throughout the observation. When it found, some insects make spores as feeding habit, but other insects feeding habit is unknown.

The presence of spores in crop and feces of insect becomes one of the indicators that insect was eating spores while visiting sporocarp of Ganoderma. Some dominant family of coleopteran also detected consuming Ganoderma spores during a visit. While all *Mycodrosophila* flies are visiting sporocarps for eating spores. *S. japonicum* is the most common insects found visiting sporocarp either in Kakuma or town. However, *S. japonicum* is not known whether it has a

noticeable effect on the spread of spores. *M. gratiosa* is the species with the highest individual detected consume Ganoderma spores.

Throughout observation lasts from May to November, insects present in sporocarp of Ganoderma seen in town and Kakuma. Seasonal change is not uniform throughout the observation, causing the variation number of insects that visit on sporocarp. The seasonal change also affects the number of insects that visit on each sporocarp. There are certain months in which number of insects present in sporocarp higher than other months.

At a particular time, sporocarp will release spores with different numbers and sizes. We also observed the effect of spores production and spore size released by sporocarp with the number of insects is present at that time. The spores release (presence and absence of spores) from sporocarp affect the insect visits on the sporocarp.

There are times where sporocarp release high spores and insect visits to the sporocarp are also high. However, there is also a pattern that sporocarp releases large spores, but the few insect was visiting sporocarp. The number of insect individual captured from sporocarps did not affect the size of the spores. There is no significant relationship if the insect is more like spores with a large size or small one. The above description also indicates that, insects visiting sporocarp not only for feeding (spores) but perhaps also as a place to stay, this is consistent with Graves (1959) in Lim (1977) that the fruiting bodies of Ganoderma is a typical wooden shelf fungus, ideal sub-strata for number of insects and other creatures, for they provide shelter, direct or indirect source of food or breeding places.

CHAPTER 4
GENERAL DISCUSSION

4.1. Author summary

Fungi have a significant role in the ecosystem. They obtain their nutrients by feeding or parasitizing all types of substrates and crucial role in the recycling of minerals and carbon by the decomposition of organic debris and waste.

Fungal spores play the primary role in both survival of a fungal species under unfavorable conditions and dispersal into habitats (Kramer 1982). Fungal spores have a unique role in fungal life cycles as they provide the genetic link between one generation and another. Spores can be part of asexual or sexual reproductive cycles, and are sometimes borne by multicellular sporocarps (Moore-Landecker 2011). Fungal spores are tiny and lightweight. The Wind carry mature spore to vicinity place. Fungal spores are released and distributed in varied ways and have a certain climatic condition.

Size and shape of spores are widely used to indicate the characteristic of fungi taxonomy for species determination, and some genera (Parmasto and Parmasto 1987 in Kauserud et al. 2008). Spore size and shape are depending on the optimization through natural selection. In general, the larger spores tend to have higher fitness, but it is more expensive to produce. It has evidenced that spore fall under gravity is the only factor needed to be invoked. Larger spores may not consistently have higher fitness, mainly due to aerodynamic constraints. For example, heavy spores fall faster than lighter ones, and this will support small spores for more efficient of wind deployment (Stearns 1992 in Kauserud et al. 2008). Spore form is potentially crucial because it will affect the shape of the spores aerodynamic properties. Spherical spores probably have the ability to gain higher speed, and thus, better to insert themselves into an object (tree bark and branches) compared to narrow spores (Deacon 1997 in Kauserud et al. 2008). On the other hand, small spores may better float through the air and, therefore, has the advantage of an increase in the deployment of wind. Spore shape also affects surface to volume ratio, which may be one of many critical factors in the survival

or reproduction of the fungus investment. *G. applanatum* have tiny hymenial pore (0.01 cm wide) with longer tubes, thus, the spores might be expected that many spores would become stranded on the pore surface before emerging into the free air below the sporophore.

4.2. Concluding remarks

Most of insects gathering on sporocarps are exclusively feeding on in spores and more than half of spores appeared intact in their feces. A variety of insects may be involved in the spore dispersal. Spore feeding insects may help the fungi from spores attaching to the hymenial surface by feeding and cleaning on the pore surfaces. Digestion of spores by *D. picta* did not retard nor promote germination rate. It was observed in *Mycodrosophila* flies as well (Shimotoku and Tuno unpubl. Data). It was taken that insect' digestion did not break spore dormancy. Only *Mycodrosophila* shows positive relation with spore size that may relate to that they consume the coating material of spores. The bigger spore could be netter food for them. While beetles destroy wall of spores to consume protoplasms. Spore size varied in time and in different sporocarps. The difference was not categorical but was more continuous. The survival and dispersal strategy of *G. applanatum* can be variable even within a genet according to its substantial spore size variation.

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