食菌性害虫ヒラタチャタテ

Liposcelis bostrychophila Badonnel の

摂食嗜好性を決定する糸状菌の特性に関する研究

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Fungal characteristics determining the fungal feeding preferences of the fungivorous pest, booklice (*Liposcelis bostrychophila* Badonnel)

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要　旨

ヒラタチャタテは屋内性チャタテムシの代表種であり、一般家屋だけでなく、食品関連施設や製薬工場などの環境に普通に見られる。また、保存された食品や穀物に好んで生息するため、世界中で保存食品の害虫として、食品衛生上深刻な問題を引き起こしている。ヒラタチャタテは体長 1.0〜1.3 mm で、成虫になっても翅がないタイプのチャタテムシである。また、雌のみで単為生殖し、生涯で約 110 個の卵を産むため、非常に高い繁殖能力を持っている。さらに、食物がなくても最大 2 ヵ月間生存可能で、屋内環境でも短期間で定着できる。食性は雑食性であるが、特にビール酵母や糸状菌を好むことが報告されている。食品関連施設や製薬工場などの施設環境に繁殖した糸状菌にヒラタチャタテなどの食菌性チャタテムシが大量発生し、体表や糞を介して環境に糸状菌を拡散させる事例が非常に多い。したがって、施設内に繁殖した糸状菌が増加すると、環境に生息するチャタテムシの数も増加するため、これらのチャタテムシは糸状菌の繁殖の指標となっている。また、ヒラタチャタテは多種の殺虫剤に耐性を持ち、その防除が困難となるため、施設内の糸状菌の繁殖を防いで、チャタテムシの発生を予防する対策が基本となる。チャタテムシの発生予防には、嗜好性が高い糸状菌の種類を理解することが重要であるが、ヒラタチャタテの糸状菌の嗜好性や、嗜好性が高い糸状菌の特性に関する研究はみられない。一方で、ヒラタチャタテなどの殺虫剤抵抗性昆虫を防除するための効果的な手段として、物理的対策や環境的対策などを統合した防除手法である総合的害虫管理（Integrated Pest Management, IPM）が最近注目されている。IPM の基本は、対象となる害虫の発生状況をモニタリングし、適切な対策を早期に行うことであり、このモニタリングを効果的に行うためには、害虫を誘引する有効な物質や手段が求められる。これまでもヒラタチャタテを効果的に捕獲できるトラップを開発するために、紫外線や餌を使った誘引剤について様々な研究がなされてきたが、摂食嗜好性が高い糸状菌をモデルとして、

その嗜好性に関する要因を多角的に解明し、誘引へ応用した研究はみられない。

そこで、本研究では食品関連施設で見られる代表的な糸状菌を対象に、ヒラタチャタテの摂食嗜好性を評価し、嗜好性の高い糸状菌の形態的特性、光学的特性、生化学的特性、糸状菌によって産生される臭気物質などの要因とそれらの関連性について検討した。本研究の概要は以下のとおりである。

Ⅰ．糸状菌に対する摂食嗜好性評価

室内環境から採取した4種の糸状菌、すなわち*Aspergillus tubingensis*、*Aspergillus flavus*、*Penicillium chrysogenum*、*Cladosporium cladosporioides*を供試菌として、ヒラタチャタテの摂食嗜好性試験を行った。供試菌を発育させた直径 8 ㎜ペーパーディスク（糸状菌ディスク）を直径 9 cm のプラスチックケースに等間隔で配置し、成虫 30 頭をケース中央から放虫した後、装置を 27±1℃（湿度 80％以上）に設定したインキュベーターに保管した。保管後 2～4 日目に各ディスク上に集まった個体数を毎日計測し、3 日間の合計数を比較した（n=10）。その結果、供試菌のなかで、最も嗜好性の高かった糸状菌は *P. chrysogenum* であった。次いで、*C. cladosporioides*、*A. tubingensis* と続き、*A. flavus* の嗜好性が最も低かった。また、*P. chrysogenum* と *C. cladosporioides* のディスク上の菌糸や胞子は摂食され、菌体はほとんど残っていなかったが、*A. tubingensis* と *A. flavus* の菌体は摂食されていなかった。これらの結果から、ヒラタチャタテは糸状菌の種類によって異なる摂食嗜好性を示すことが明らかとなり、*P. chrysogenum* の嗜好性が最も高いことが判明した。

Ⅱ．摂食嗜好性に関わる糸状菌の形態的、光学的および生化学的特性

嗜好性評価試験において最も嗜好性が高かった *P. chrysogenum* の形態的、光学的、生化学的特性について、供試菌の他 3 種と比較し、ヒラタチャタテの摂食行動と、嗜好性を決定する糸状菌の特性との関連性を調

べた。まず、形態的特性について、糸状菌コロニーの表面特性および高さ、胞子の形状およびサイズ、菌糸の太さを拡大観察した結果、*P. chrysogenum* と *C. cladosporioides* のコロニー表面は滑らかで、菌糸が密に発育したビロード状であった。一方、*A. tubingensis* と *A. flavus* のコロニー表面は不均一で、菌糸の密度が粗い粉状であった。*P. chrysogenum* と *C. cladosporioides* のコロニーの高さは 200 µm、*A. flavus* は 400 µm、*A. tubingensis* は 1,000 µm であり、高さに大きな差が見られた。ヒラタチャタテは、糸状菌の摂食時に、脚を曲げ、口器を糸状菌の位置まで下げて菌体を顎で剥し取っていた。ヒラタチャタテの体高は 300～400 µm で、口器は頭部の下面に位置し、頸部の駆動域が小さいため、口器よりも低い位置の餌を摂食しやすい体の構造となっていた。*P. chrysogenum* および *C. cladosporioides* のコロニーの高さは、ヒラタチャタテの体高よりも低かった。また、菌糸と胞子は、ヒラタチャタテの口器よりも低い位置で密集して発育していたため、これらの菌体を容易に食べることができた。これらの結果より、ヒラタチャタテの糸状菌摂食時の行動が明らかとなり、この摂食行動の詳細については世界で初めての報告である。さらに、ヒラタチャタテは、コロニーの高さが低いビロード状の糸状菌である *P. chrysogenum* および *C. cladosporioides* を餌として好むことが判明した。

次に、糸状菌を UV 照射（330～385 nm）下で観察した結果、*A. tubingensis* と *A. flavus* の菌糸は発光したが、摂食嗜好性の高い *P. chrysogenum*、*C. cladosporioides* の菌糸は発光せず、発光しない糸状菌の方が、嗜好性が高いことが示唆された。さらに、糸状菌ディスクの pH を測定した結果、*P. chrysogenum* を含む他の供試菌は 5.2～6.6 であったが、*A. tubingensis* は 3.1 と酸性を示した。*A. tubingensis* は嗜好性評価試験においては、2 番目に嗜好性が高かったが、ディスクの菌体は摂食されずに残っていたことから、糸状菌を餌としてではなく、虫体よりも高い菌糸のコロニーを潜伏場所としていたことが推察された。

Ⅲ. 糸状菌の臭気による嗜好性評価および臭気物質の同定

糸状菌が産生する臭気物質による嗜好性について、自作の嗅覚誘引装置を用いて評価を行った。装置は中央ボックスと 4 つのチャンバーで構成され、各チャンバーに 4 種の糸状菌ディスクを配置し、中央ボックスに向かって、各チャンバー側から空気が流れるように調整した。成虫 120 頭を中央ボックスから放虫し、装置を 27±1℃（湿度 80％以上）に設定したインキュベーターに保管した。保管後 3 日目に各チャンバー内の個体数を計測した（n=5）。その結果、*P. chrysogenum* の糸状菌ディスクを置いたチャンバーで最も多くの個体数が確認された。次いで、*C. cladosporioides*、*A. flavus* が多く、*A. tubeingensis* は誘引された個体数が最も少なかった。摂食嗜好性評価の結果と同様に、臭気による評価試験においても *P. chrysogenum* が高い嗜好性を示し、ヒラタチャタテの摂食行動を誘発させる臭気物質の産生が示唆された。

そこで、*P. chrysogenum* が産生した臭気物質を同定するため、各糸状菌ディスクの臭気物質を GC/MS で測定した結果、35 種類の臭気物質が検出された。嗜好性が最も高かった *P. chrysogenum* から検出された物質は 9 種類（styrene、1,5-octadien-3-ol、1-octen-3-ol、3-octanone、4-methylundecane、2,6,10-trimethyldodecane、3,3,4-trimethyldecane、3,4,5,6-tetramethyloctane、butylated hydroxy toluene）であり、このうち 1,5-octadien-3-ol と 2,6,10-trimethyldodecane は、*P. chrysogenum* のみから検出された。したがって、これらの臭気物質がヒラタチャタテの誘引物質の候補であることが示唆された。

このように、本研究では、ヒラタチャタテの糸状菌の摂食嗜好性試験の結果により、糸状菌の種類によって嗜好性が異なり、食品関連施設で一般的に見られる 4 種の糸状菌の中では、*P. chrysogenum* が最も嗜好性が高いことが明らかになった。また、ヒラタチャタテの嗜好性が高い糸状菌の特性との関連性を検討し、摂食嗜好性を決定する糸状菌に共通する要因を特定した。すなわち、ヒラタチャタテにとって嗜好性の高い糸

状菌は、口器よりも低い位置で密集して発育するビロード状のコロニーを形成し、また紫外線照射下で菌糸は発光しない種類であった。さらに、これらの糸状菌は特定の臭気物質を産生し、ヒラタチャタテは摂食嗜好性の高い糸状菌の臭気を探知できるものと考えられた。

これらの結果により、食品工場や食品貯蔵施設、製薬工場など多くの施設で問題となっているチャタテムシの防除において、チャタテムシの繁殖リスクが高い糸状菌の種類を把握することの有効性が示された。また、こうした糸状菌をモニタリングすることにより、チャタテムシの発生リスクを早期に予測することができる。さらに、施設環境に存在するリスクの高い糸状菌を積極的に除去することができれば、チャタテムシをはじめとする食菌性害虫の発生予防につながると考えられた。一方で、本研究で明らかになった糸状菌が産生する特定の臭気物質など、嗜好性の高い糸状菌がもつ共通の特性は、チャタテムシの効率的なモニタリングおよび捕獲に必要な誘引剤の開発などに応用が可能であり、世界的に検討が進められているチャタテムシに対する総合的有害生物防除（IPM）の手法の発展につながると考えられた。

Abstract

Liposcelis bostrychophila (booklice) are a representative species of indoor psocids that are commonly found in ordinary houses and environments such as food-related facilities and pharmaceutical factories. As pests that affect preserved foods all over the world, they cause serious food hygiene problems because they prefer to live in preserved foods and grains. Booklice are 1.0 to 1.3 mm in body length and are a type of psocid with no wings, even upon reaching adulthood. Furthermore, they reproduce parthenogenetically with only females; as a single booklouse lays approximately 110 eggs in her lifetime, they have an extremely high breeding capacity. They can survive for up to two months without food and can become established in indoor environments within a short period of time. Although they are omnivorous, it has been reported that they particularly prefer brewer's yeast and fungi. There are many cases of huge outbreak of fungivorous psocids, such as booklice, occurring in fungi that have propagated in

institutional environments, such as food-related facilities and pharmaceutical factories, leading to the spread of fungi in the environment via body surfaces and feces. Because the number of psocids inhabiting the environment also increases, as the propagation of fungi increases in the facility, these psocids are indicators of fungal development. Since booklice are resistant to various insecticides and difficult to control, basic measures include preventing outbreaks of psocids by preventing the development of fungi in the facility. Although an understanding of the types of fungi strongly preferred by these pests is important for preventing outbreaks of psocids, there have been no studies on the fungal preferences of booklice or of the characteristics of fungi that are strongly preferred. Integrated pest management (IPM), which is a control method that integrates physical and environmental measures, has recently gained attention as an effective means of controlling insecticide-resistant insects, such as booklice. The basis of IPM involves monitoring the outbreak of the target pests and taking appropriate measures at an early stage. Effective substances and means for attracting pests are required to effectively carry out the

monitoring. Various studies have been conducted on attractants using ultraviolet rays and bait in order to develop traps that can effectively capture booklice. However, to our knowledge, no studies have determined the factors related to these insects' preferences and applied them to attraction using fungi of high feeding preference as a model.

Therefore, in the present study, we evaluated the feeding preferences of booklice concerning typical fungi found in food-related facilities and investigated the morphological, optical, and biochemical profiles of the highly preferred fungi as well as factors, such as odorous substances, produced by these fungi and their relationship to the preference. The study outline is shown below.

Ⅰ. The evaluation of fungal feeding preferences

A booklouse feeding preference test was conducted using four species of fungi collected from an indoor environment, namely *Aspergillus tubingensis*, *Aspergillus flavus*, *Penicillium chrysogenum*, and *Cladosporium cladosporioide.* Paper discs (fungus discs) of 8 mm in diameter on which the test fungi were grown were placed at equal intervals in

a plastic case 9 cm in diameter. Thirty adult booklice were released from the center of the case, and then the device was stored in an incubator set at 27±1 °C (humidity ≥80%). The population gathered on each disc was counted daily on the 2nd to 4th days of storage, and then the total population across 3 days was compared among discs (n=10).

Among the test fungi, the booklice showed the highest preference for *P. chrysogenum*, followed by *C. cladosporioides* and *A. tubingensis*, in order, with *A. flavus* being shown the lowest preference. Although the hyphae and spores on the discs of *P. chrysogenum* and *C. cladosporioides* were eaten, leaving few remaining fungus bodies, the fungus bodies of *A. tubingensis* and *A. flavus* were not eaten. These findings indicate that the booklice showed different feeding preferences among fungi, with the highest preference shown for *P. chrysogenum*.

II. Morphological, optical, and biochemical profiles of fungi, in relation to feeding preference

The morphological, optical, and biochemical profiles of *P. chrysogenum*, which was shown the highest preference in the preference evaluation test, were compared with the other three tested species of fungi in order to identify the characteristics of the fungi that determined the feeding behavior and preferences among booklice.

First, concerning the morphological profiles, we magnified and observed the surface characteristics and height of the fungal colonies, along with the shape and size of the spores and the thickness of the hyphae. The colony surface of *P. chrysogenum* and *C. cladosporioides* were found to be smooth and velvety with densely grown hyphae, whereas the surfaces of *A. tubingensis* and *A. flavus* colonies were uneven, and the hyphal density was coarse and powdery. The heights of the colonies of *P. chrysogenum* and *C. cladosporioides* were 200 μm, while *A. flavus* was 400 μm, and *A. tubingensis* was 1,000 μm, indicating a large difference in height. When booklice ate the fungus, they bent their legs and lowered their trophi to the position of the fungus, then peeled the fungus bodies off with their jaws. The height of the booklice was 300 to 400 μm, the trophi were located

on the underside of the head, and because the thrusting range of the neck is small, the body structure was such that it was easy to eat food at a position lower than the trophi. The heights of the colonies of *P. chrysogenum* and *C. cladosporioides* were lower than the height of the booklice. In addition, because the hyphae and spores grew densely at a position lower than the trophi of booklice, it was easy for them to eat these fungus bodies. Based on these results, the behavior of booklice while eating fungi was clarified. To our knowledge, this is the first report on the details of this feeding behavior. These findings indicate that booklice prefer velvety fungi, such as *P. chrysogenum* and *C. cladosporioides*, with a low colony height.

Next, upon observing the fungi under UV irradiation (330–385 nm), while the hyphae of *A. tubingensis* and *A. flavus* emitted light, the hyphae of *P. chrysogenum* and *C. cladosporioides*, which had been shown to have a high feeding preference, did not emit light, suggesting that the fungi that did not emit light had higher preference.

Furthermore, the pH of the fungus disc of *A. tubingensis* was 3.1, while those of

other test fungi, including *P. chrysogenum*, ranged from 5.2–6.6, indicating that *A. tubingensis* was acidic. It was thus assumed that *A. tubingensis,* which has hyphae that are taller than the body height of booklice, was used as a hiding place, rather than for feeding, as the fungal bodies of the disc remained uneaten despite *A. tubingensis* being the second-most preferred fungus in the preference evaluation test.

III. The evaluation of preference by odor of fungi and identification of odorous substances

The preference of the odorous substances produced by the fungi was evaluated using a handmade olfactometer device. The device consisted of a central box and four chambers, with four species of fungus discs placed in each chamber, and the air was adjusted to flow from each chamber side toward the central box. One hundred and twenty adult booklice were released from the central box, and the device was stored in an incubator set at 27±1 °C (humidity ≥80%). The population in each chamber was measured on the 3rd

day of storage (n=5). As the result, the largest population was confirmed in the chamber in which the fungus disc of *P. chrysogenum* had been placed, followed by *C. cladosporioides* and *A. flavus*, in order, with *A. tubingensis* having attracted the fewest booklice. Similar to the results of the feeding preference evaluation, the booklice showed a high preference for *P. chrysogenum* in the odor evaluation test, suggesting the production of odorous substances that induced feeding behavior among booklice.

Next, to identify the odorous substances produced by *P. chrysogenum*, the odorous substances of each fungus disc were measured by GC/MS, and as a result, 35 types of odorous substances were detected. Nine substances were detected in *P. chrysogenum*, which had the highest palatability (styrene, 1,5-octadien-3-ol, 1-octen-3-ol, 3-octanone, 4-methylundecane, 2,6,10-trimethyldodecane, 3,3,4-trimethyldecane, 3,4,5,6-tetramethyloctane, and butylated hydroxytoluene). Among these, 1,5-octadien-3-ol and 2,6,10-trimethyldodecane were detected only in *P. chrysogenum*, suggesting that these odorants are candidate booklice attractants.

As indicated above, this study revealed that preferences by booklice differ among species of fungus, given the results of the fungal feeding preference test of booklice, with *P. chrysogenum* being the most preferred among the four species of fungi commonly found in food-related facilities. We also examined the characteristics of fungi highly preferred by booklice in order to identify the fungal factors that determine feeding preference. Fungi that are highly preferred by booklice form velvety colonies that grow densely at a position lower than the trophi, and their hyphae do not emit light under ultraviolet irradiation. Furthermore, these fungi are believed to produce specific odorous substances that booklice can detect, allowing them to find their preferred food more easily.

These results indicate that controlling psocids will require understanding the species of fungi that are associated with a high risk of psocid breeding, which is problematic in many facilities, including food factories, food storage facilities, and pharmaceutical factories. Monitoring these fungi can predict the risk of psocid occurrence at an early stage. Furthermore, the proactive removal of such high-risk fungi present in

institutional environments may lead to the prevention of outbreaks of fungivorous pests, such as psocids. Determining the common properties of highly preferred fungi, such as the specific odorous substances produced by the fungi that were revealed in this study, can be used to improve the efficiency of monitoring of psocids and help develop attractants to capture these pests. This may lead to the development of integrated pest management methods for psocids, which are currently being studied worldwide.

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Multifaceted fungal characteristics determining the fungal feeding preferences of the psocid, *Liposcelis bostrychophila* badonnel (Psocoptera: Liposcelidae)

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ABSTRACT

Psocids inhabit stored foods and cereals and cause serious problems with regard to food hygiene. They are a major target for pest control around the world, including Japan. *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelidae) is a representative species of household psocid that prefers to feed on fungi while also spreading fungi in the site it inhabits; it is therefore recognized as an indicator of fungal generation. The present study investigated the characteristics of fungi that influence the feeding behavior of this fungivorous insect using four fungus species (*Aspergillus tubingensis*, *Aspergillus flavus*, *Penicillium chrysogenum*, *Cladosporium cladosporioides*) that are representative fungi found in Japanese food factories and food storage facilities. The results of preference and feeding tests performed using an arena device (diameter: 9 cm) and a handmade olfactometer revealed that *P. chrysogenum* was the most attractive fungus for feeding. Based on the comparison of multiple characteristics of *P. chrysogenum* and the three other fungus species, the factors that were considered to evoke the feeding behavior of *L. bostrychophila* were a velvety appearance with hyphae that were shorter than the height of the insect as well as specific odors. The present study clarified the common characteristics of the fungi preferred by *L. bostrychophila*, which may be used to predict pest occurrence and develop agents for trapping and monitoring, thus leading to effective integrated pest management.

1. Introduction

Several species of psocids commonly inhabit stored foods and grains and thus cause serious problems in relation to food hygiene (Turner, 1994; Athanassiou et al., 2010). Especially in food-related facilities and pharmaceutical factories, fungivorous psocids, such as *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelidae), can occur from fungi in a facility environment (Tani and Ito, 2006; Trematerra and Catalano, 2010). These insects not only cause the contamination of stored foods, processed foods, and pharmaceuticals with foreign bodies, but also their carcasses and feces are allergens that affect human health (Tani and Ito, 2006; Fukutomi et al., 2012; Nayak et al., 2014; Hubert et al., 2018). For these reasons, they have become a major target for pest control in households, food stores, grain storage, food factories and pharmaceutical factories (Turner, 1994; Rajendran, 1994; Ahmedani et al., 2010; Tani and Ito, 2006).

L. bostrychophila is one of the most common types of psocid inhabiting households and it is widely distributed around the world, including Japan (Howard and Lord, 2003; Green and Turner, 2005; Fukutomi et al., 2012; Rees, 1994; Wang et al., 1999; Ding et al., 2002). The adult insect is wingless and small, with a body length of 1.0–1.3 mm and a brown body color (Fukutomi et al., 2012). They live for up to two months without food, and the female insects are thelytokously parthenogenetic (Tuner and Maude-Roxby, 1988; Howard and Lord, 2003). The mature female lays approximately 110 eggs in her lifetime, so *L. bostrychophila* has an extremely high breeding capacity and is rapidly established in indoor environments (Turner, 1994). The insect is generally polyphagous, but it has been reported to prefer brewer's yeast and fungi (Mills et al., 1992; Green and Turner, 2005; Green, 2008). Turner (1994) reported that the preferred living environment of

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L. bostrychophila was a temperature of 20–30 °C and 65%–80% humidity, which is also a condition that allows many fungi to grow easily. Since the insect has organs that can maintain fungi on the cuticle surface of the body and excretes live fungi in its feces, it can contribute to the diffusion of microorganisms into the environment (Tani and Ito, 2006; Kalinovic et al., 2006). Once the fungi begin to grow in facilities in which the insect lives, the fungal cycle begins to rapidly increase (Mills et al., 1992). For these reasons, these organisms are considered to be an indicator of fungal development, and the number of insects inhabiting an environment has been reported to vary depending on the amount of fungi generated (Tani and Ito, 2006). In order to control these insects, it is important to understand their fungal feeding preferences. The feeding preferences for certain fungivorous insects and stored-grain insects have been reported (Sinha, 1971; Wright et al., 1980), but there are few studies on the preferences of *L. bostrychophila* and the fungal factors that are attractive to them.

Recently, integrated pest management (IPM) has been attracting attention as an effective means for the control of *L. bostrychophila*. IPM is the integrated combination of various measures such as environmental and physical extermination measures. Since it is thought to be an effective means for insecticide resistant pests, such as psocids, multifaceted measures are being studied as an approach to IPM. To reduce the insect population, capture and monitoring methods using traps are one of the underlying tools (Leong and Ho, 1994; Green and Turner, 2005; Ahmedani et al., 2010; Diaz-Montano et al., 2014, 2015, 2016, 2018). Focusing on L. bostrychophila, some studies have revealed that exposure to limited ultraviolet (UV) wavelengths and maintaining wheat at different temperature gradients were attractive ways of achieving effective IPM (Diaz-Montano et al., 2016; Green, 2009; Throne and Flinn, 2013). However, no information has been gathered regarding the fungi feeding preference of *L. bostrychophila*. In order to develop traps that can effectively capture these pests, methods of attracting and using bait have thus been studied. Focusing on *L. bostrychophila*, some studies have revealed that exposure to limited ultraviolet (UV) wavelengths and maintaining wheat at different temperature gradients were attractive ways of achieving effective IPM (Diaz-Montano et al., 2016; Green, 2009; Throne and Flinn, 2013). However, no information has been gathered regarding the fungi feeding preference of *L. bostrychophila*.

The present study investigated the fungi most preferred as feed for *L. bostrychophila* and assessed the factors related to feeding preference. We examined the feeding preferences among four common fungi isolated from food factories and food storage facilities in Japan (*Aspergillus*, *Penicillium*, *Cladosporium*) and analyzed the morphological, biochemical and optical profiles as well as the odorous substances produced by the fungi. The data obtained from this study may also be used to establish effective IPM control measures, such as tools to monitor and capture *L. bostrychophila* in food factories and food storage facilities.

2. Materials and methods

2.1. Insects

Liposcelis bostrychophila was purchased from an insect supplier (VIABLE, Ltd., Hyogo, Japan). The insects were cultured in plastic culture cases (diameter, 9 cm; height, 4.5 cm height; volume, 350 cm^3) with a 1:1 mixture of milled laboratory animal feed (CE-2; Clea Japan, Inc., Tokyo, Japan) and brewer's yeast powder (Asahi Group Foods, Ltd., Tokyo, Japan). Two plastic cases were placed inside a plastic box (20 × 13.6 × 6.8 cm; 1850 cm^3 volume) with a saturated sodium chloride solution that maintained the humidity at >80%. The plastic boxes were kept in an unlit incubator at a constant temperature of 27 ± 1 °C.

2.2. Selection of fungi and pure fungal cultures on discs

Four fungus species (*Aspergillus tubingensis*, *Aspergillus flavus*, *Penicillium chrysogenum*, *Cladosporium cladosporioides*) collected from indoor floors and homogenized fungivorous insects captured in a building in Narashino City, Chiba Prefecture, Japan, were used in this study. The indoor fungi were collected by the swab method from the floor, and homogenized fungivorous insects were prepared according to the method of Yoshinami et al. (2018). In brief, each individual insect was placed into a tube containing 0.1 ml of sterile phosphate-buffered saline and then homogenized and mixed with a vortex mixer for 1 min. The slurry and swab were cultured on potato dextrose ager (PDA) plates (Nissui Pharmaceutical, Tokyo, Japan) for 7 days at 25 ± 1 °C. None of the fungus strains used in this study were capable of producing mycotoxins. These fungus strains were identified based on the morphological characteristics and nucleotide sequence homology of the 28S rDNA D1/D2 region and the β-tubulin gene using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information. To prepare the fungus-cultured paper disc, each cultured fungus on PDA was collected with a sterile cotton swab, smeared onto 5 sterilized 8-mm antibiotic assay paper discs ADVANTEC® (Toyo Roshi, Ltd., Tokyo, Japan) and placed on a PDA plate. After 7 days of culturing at 25 ± 1 °C, the paper discs on which the fungi had grown were peeled from the agar with tweezers. These discs were then used for the tests in this study.

2.3. Test of the fungal feeding preferences of L. bostrychophila and observation of fungal feeding behavior

Plastic device cases (bottom diameter, 9 cm diameter; top diameter, 11 cm; height, 8 cm; volume, 625 cm^3; 9d-PDC) were used as an arena for testing fungal feeding preferences. On the bottom of the plastic case, the fungus-cultured paper disc with each of the four fungus species (*A. tubingensis*, *A. flavus*, *P. chrysogenum*, *C. cladosporioides*) and a control disc (fungus-free disc) were placed at equal intervals with a filter paper stick (Fig. 1). Thirty adult insects (mixed ages) were collected into a vial (diameter, 2.5 cm; height, 5 cm height; volume, 24.5 cm^3) from the plastic culture case and placed in the center of the plastic device case with the vial turned upside down. The plastic device case was kept in an unlit incubator at a constant temperature of 27 ± 1 °C and >80% humidity. The number of insects present on each disc was measured each day on Days 2–4 after installation, and the numbers across 3 days were totaled. The test was repeated 10 times with new paper discs each time. For this test, we observed the fungal feeding behavior of the individual insects under a stereomicroscope (S6D; Leica Microsystems GmbH, Wetzlar, Germany).

2.4. Morphological, biochemical and optical profiles of the four fungus species

The morphological, biochemical and optical profiles of the four fungus species cultured on paper discs, including the surface properties and the colony height, the shape and size of the spores, the thickness of the hyphae and the pH of the fungus, were examined. The colony height, size and shape of the spores and thickness of the hyphae were measured using a light microscope (BX41; Olympus Corporation, Tokyo, Japan) at 200 × to 400 × magnification. The hyphae and spores were observed under UV irradiation (330–385 nm) using a fluorescence microscope (BX41; Olympus Corporation). To measure the pH of the fungi, the fungus-cultured paper discs were placed in distilled water and

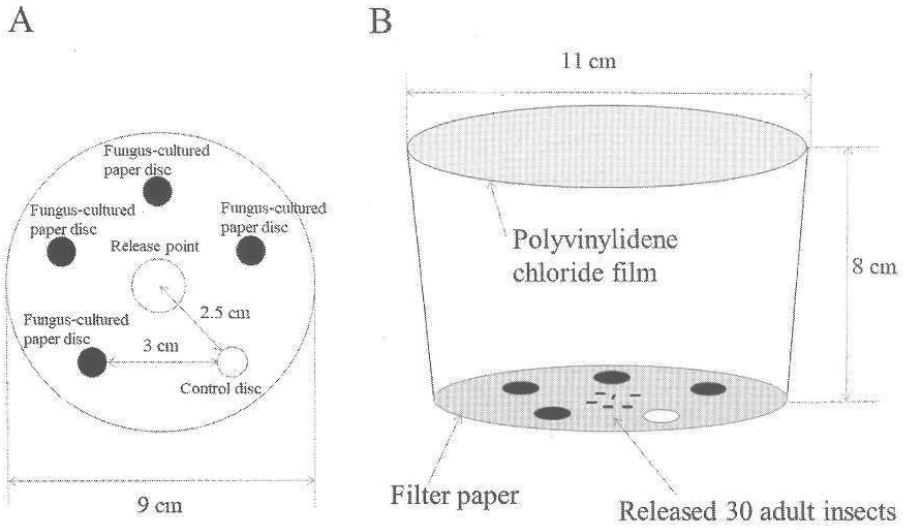

Fig. 1. Plastic device cases for the fungi preference test (cross-distance arena [diameter: 9 cm]; 9d-PDC). A: Top view, B: Bird's-eye view. The device used was a plastic case (bottom diameter, 9 cm; top diameter, 11 cm diameter; height, 8 cm; 9d-PDC) with filter paper pasted on the bottom, and the fungus-cultured paper disc with each of the 4 fungus species and a control disc were arranged at equal intervals (release point-disc, 2.5 cm; disc-disc, 3 cm). In order to make it easier to observe the fungal preferences and feeding behavior after releasing 30 adult insects into the center of the case, the upper surface was covered with a transparent polyvinylidene chloride film.

homogenized with a Biomasher® (SP; Nippi, Inc., Tokyo, Japan), and then the pH of each fungus was measured with a pH meter (LAQUA twin; Horiba Ltd., Kyoto, Japan).

2.5. Test of fungal odor preferences of L. bostrychophila

The fungal odor preferences were tested using a handmade olfactometer device (HOD), which was a modified version of the device made by Hori (2003). The HOD consisted of a central box (13 × 13 × 7.5 cm) and 4 chambers (9 × 6 × 4.5 cm), and the fungus-cultured paper discs with the four fungus species were placed on the bottom in each chamber (Fig. 2). The air flow side of each chamber was covered with activated charcoal filter to prevent other smells from flowing into the central box. The air flow in the central box was supplied by an electric fan, with the air entering from the air flow side of each chamber (mean wind speed: 0.05–0.14 m/s) and exiting into the central box.

Thirty adult insects (mixed ages) were collected into 4 vials each (diameter, 2.5 cm; height, 5 cm; volume, 24.5 cm^3) from the plastic culture case and placed in the central box of the HOD with the vials turned upside down. A total 120 adult insects were thus released. After their release, ordinary flow was maintained in the HOD under dark conditions at 27 ± 1 °C and >80% humidity at day 3 after installation, and the number of insects in each chamber was

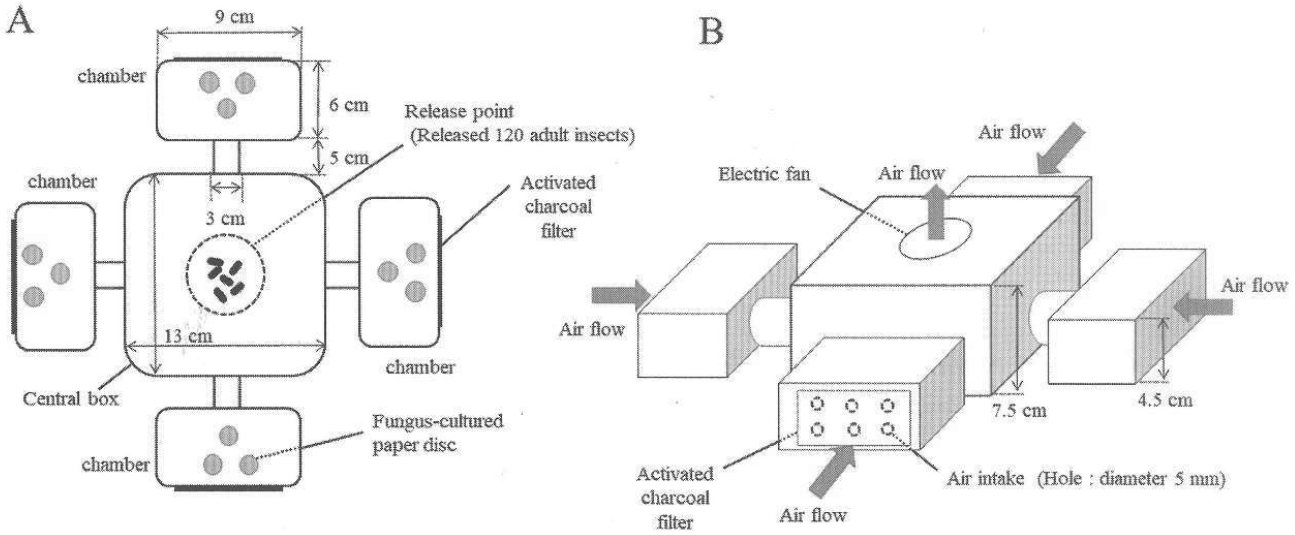

Fig. 2. Handmade olfactometer. A: Top view, B: Bird's-eye view. The handmade olfactometer device (HOD) consists of 1 central box (13 cm × 13 cm; height, 7.5 cm) and 4 chambers (9 cm × 6 cm, height, 4.5 cm) with a fungus-cultured paper disc for each of the 4 fungus species. In addition, connection paths (diameter: 3 cm × 5 cm) were installed so that the insects could move from the central box to each chamber. The device was made entirely of plastic, and filter paper was affixed to all bottom surfaces in the device, so that the insects could move easily. An electric fan was installed at the top of the central box. During the test, the fan was rotated to direct fungal odors from the fungus-cultured paper discs in each chamber toward the central box. Six holes (diameter: 5 mm) were made in the air intakes from the chamber, and an activated charcoal filter was attached to the outside.

counted. The test was repeated 5 times, with new paper discs prepared for each replication.

2.6. Measurement of odorous substances of the four fungus species

Each fungus-cultured paper disc and a control paper disc was placed into a vial, and odorants were extracted by head space solid-phase microextraction (SPME) at 35 °C for 30 min. The odorous substance was absorbed using a 50/30-μm DVB/CAR/PDMS fiber (Supelco, Bornem, Belgium), and desorption from SPME was performed for 2 min at the inlet of a GCMS-QP 2010 gas chromatograph (Shimadzu Corporation, Kyoto, Japan). The column used was a capillary column (Rtx-5MS; Restek Corporation, Bellefonte, PA, USA). The injector temperature was 250 °C, the transfer line temperature was 250 °C, and the oven temperature was started at 35 °C, held for 5 min, and programmed to increase from 35 to 120 °C at 5 °C/min. The odorous substances were identified by comparing the obtained mass spectrum to the mass spectrum library in the Wiley Registry of Mass Spectral Data 10th Edition (Shimadzu Corporation).

2.7. Data analyses

The test for the fungal feeding preferences was repeated 10 times, and the test for the fungal odor preferences was repeated 5 times. All results were expressed as the mean and standard error. In addition, to compare the data from four fungus species of the preference tests, the Steel-Dwass test (SD test) for multiple comparisons (KyPlot software program version 5.0; KyensLab Inc., Tokyo, Japan) was used. Probability values of $P < 0.05$ were considered to indicate statistical significance.

3. Results

3.1. Fungal feeding preferences of L. bostrychophila

To examine the fungal feeding preferences in a close-distance arena, the test was performed using the 9d-PDC. In the 9d-PDC, the released insects could select their preferred fungi based on multiple factors, such as the visual, optical, biochemical and olfactory characteristics.

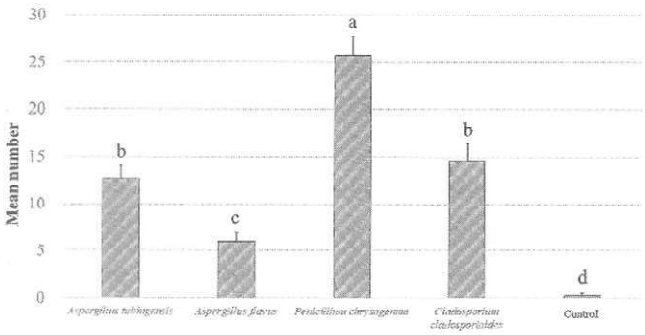

Fig. 3. Number of L. bostrychophila that selected each of the four fungus species in the plastic device cases (9d-PDC). Thirty adult insects were released into the center of the plastic device case (diameter: 9 cm; 9d-PDC) and observed for 4 days. From Days 2–4, the number of insects present on each disc was measured daily, and finally, the mean of total insect number was calculated. The number of insects on the fungus-cultured paper disc with each of the four fungus species was compared, with mean and standard error values as follows: *A. tubingensis*: 12.7 ± 1.3, *A. flavus*: 6.0 ± 0.9, *P. chrysogenum*: 25.7 ± 2.0, *C. cladosporioides*: 14.6 ± 1.8. Different letters indicate significant differences in the mean of total insect number, determined using a Steel-Dwass test at $P < 0.05$ (n = 10). Error bars represent the standard error.

Fig. 3 shows the results of the fungal feeding preferences using the 9d-PDC. Of the four species, the most preferred fungus was *P. chrysogenum*; this preference was statistically significant (SD test, $P < 0.05$). The second-most preferred fungi were *C. cladosporioides* and *A. tubingensis*, and the difference between these species was not statistically significant (SD test, $P > 0.05$). *A. flavus* was the least preferred species, with a statistically significant difference between *A. flavus* and the other species (SD test, $P < 0.05$). By day 4 after release, although many hyphae and spores remained on the *A. tubingensis* and *A. flavus* disks, the *P. chrysogenum* and *C. cladosporioides* disks retained almost no hyphae or spores (data not shown).

3.2. Morphological, optical and biochemical characteristics of the four fungus species and observation of fungal feeding behavior

To determine why *P. chrysogenum* was the most preferred by *L. bostrychophila* in the 9d-PDC-test, we morphologically, optically and biochemically compared *P. chrysogenum* with the other three fungus species. Table 1 shows the morphological and biochemical characteristics of the four fungus species grown on paper discs. The macroscopic appearance of *P. chrysogenum* and *C. cladosporioides* was velvety, showing a low height, smooth surface and dense compaction, while that of *A. tubingensis* and *A. flavus* was powdery, uneven and rough. The heights of *P. chrysogenum* and *C. cladosporioides* colonies were approximately 200 μm, while those of *A. tubingensis* and *A. flavus* were 1000 and 400 μm, respectively (Fig. 4). The *P. chrysogenum* spores measured 2–4 μm in size and spherical in shape, while the *A. tubingensis* and *A. flavus* spores measured 3–5 μm in size and spherical in shape. In contrast, *C. cladosporioides* spores measured 3 × 7–8 μm in size with an ellipsoidal and/or spindle shape. The thickness of the hyphae of *P. chrysogenum* and *C. cladosporioides* was 3–4 μm, while that of *A. tubingensis* and *A. flavus* was 10–15 μm and 4–5 μm, respectively. In addition, on observing how the adult insects ate the fungi, the fungi were found to be peeled off with the jaws and chewed (Fig. 5).

Microscopic observation of the hyphae and spores of the four species of fungi under UV irradiation (330–385 nm) revealed that the hyphae of *A. tubingensis* and *A. flavus* had luminescence, while those of *P. chrysogenum* and *C. cladosporioides* did not (Fig. 4).

Regarding the biochemical profile, the pH of the homogenized solutions of the four fungi were measured (Table 1). The pH of *A. tubingensis* was the lowest, at 3.1, while the pH of the other fungi, including *P. chrysogenum*, ranged from 5.2 to 6.6. The fungal body and metabolites of *A. tubingensis* were strongly acidic.

3.3. Fungal odor preferences of L. bostrychophila

The effect of fungal odor on food-seeking behavior was examined using an HOD. In the HOD, the released insects could not see the fungi directly and had to select fungi based on their odor alone. As shown in Fig. 6, the largest number of insects were detected in the chamber containing *P. chrysogenum*; the number of insects in this chamber was significantly higher than in the other three species (SD test, $P < 0.05$). The chambers containing *C. cladosporioides* or *A. flavus* gathered the second and third most insects, respectively (SD test, $P < 0.05$), but there was no significant difference between these two species (SD test, $P > 0.05$). The chamber containing *A. tubingensis* contained significantly fewer insects than the chambers containing the other three species; (SD test, $P < 0.05$). Similar to the 9d-PDC results, the most attractive fungal odor was that of *P. chrysogenum*. These results suggested that *P. chrysogenum* produced the most attractive odor for evoking feeding behavior.

Table 1
The morphological and biochemical characteristics of four fungus species cultured on paper discs.

Fungus species	Colony[a]		Spore[b]		Hyphal thickness[c] (μm)	pH
	Surface properties	Height(μm)	Shape	Size (μm)		
Aspergillus tubingensis	powdery	1000	globose	3–5	10–15	3.1
Aspergillus flavus	powdery	400	globose, subglobose	3–5	4–5	6.2
Penicillium chrysogenum	velvety	200	subglobose, ellipsoidal	2–4	3–4	5.2
Cladosporium cladosporioides	velvety	200	ellipsoidal, spindle	3x7-8	3–4	6.6

[a] The characteristics of the fungal colonies cultured on paper discs; the surface property and the height are presented. The height of the fungus colonies is shown in Fig. 4.
[b] The main spore shape and size produced by the fungus are presented. The size is presented as the range in spore diameters; spindle-shaped spores are shown as the range of the minor axis × major axis.
[c] The thickness of the hyphae are expressed as the range in diameter.

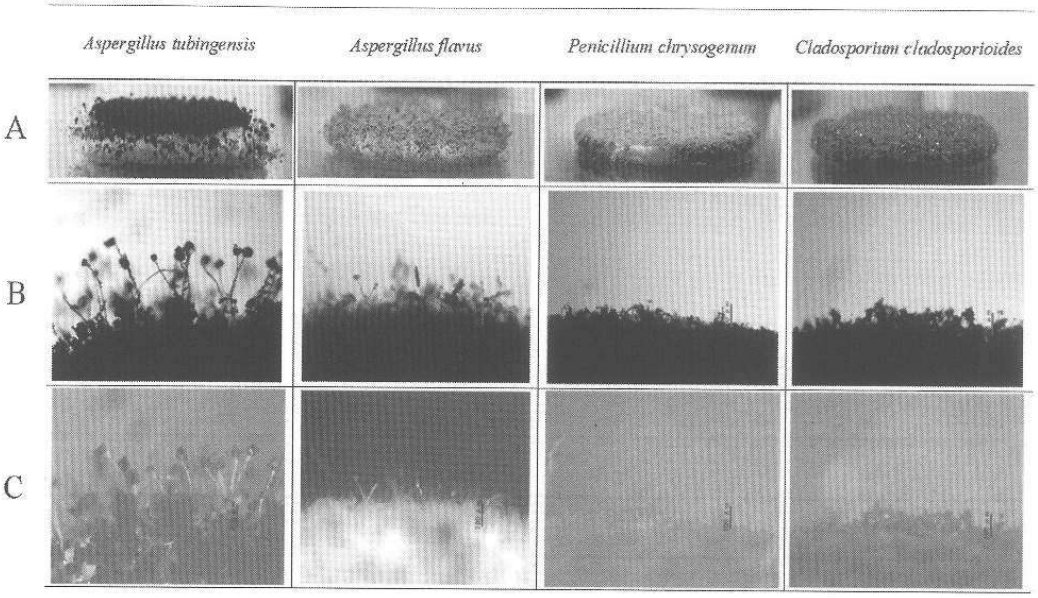

Fig. 4. Morphological and optical profiles of the four fungus species cultured on paper discs. Each fungus was cultured on a paper disc for 7 days at 25 °C. A: Fungi cultured on paper discs. B: The height of the cross-section was measured using a light microscope at 400 × magnification (scale bar = 200 μm). C: The fungi were observed under ultraviolet irradiation (330–385 nm) with a fluorescence microscope at 400 × magnification (Scale bar = 200 μm).

Fig. 5. The fungal feeding behavior of L. bostrychophila. The behavior of *L. bostrychophila* eating *P. chrysogenum* was observed under a stereomicroscope. The legs were bent on the fungus, the head was lowered, and the trophi was moved to the position of the fungus. The fungus was peeled off with the jaws of the trophi and then chewed.

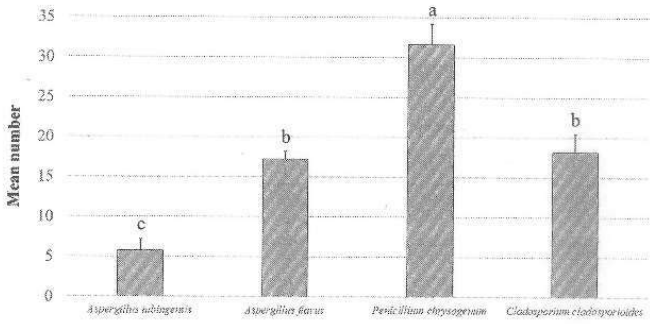

Fig. 6. Number of L. bostrychophila specimens that selected each of the four species in the handmade olfactometer device (HOD). One hundred and twenty adult insects were released into the HOD and observed for 3 days. On day 3, the number of insects at each fungus was counted, and the numbers were compared, with mean and standard error values as follows: *A. tubingensis*: 5.8 ± 1.4, *A. flavus*: 17.2 ± 1.0, *P. chrysogenum*: 31.6 ± 2.6, *C. cladosporioides*: 18.2 ± 2.2. Different letters indicate significant differences in the mean of insect number, determined using a Steel-Dwass test at $P < 0.05$ (n = 5). Error bars represent the standard error.

3.4. Odorous substances of fungi that affect feeding behavior

To determine which attractive odors produced by fungi influence feeding behavior, fungal odor substances were identified by gas chromatography/mass spectrometry (GC/MS). Thirty-five representative odorous substances and mass spectra detected from the four fungus species are listed in Table 2 and Fig. S1. Of the

35 compounds. 9 (styrene, 1,5-octadien-3-ol, 1-octen-3-ol, 3-octanone, 4-methylundecane, 2,6,10-trimethyldodecane, 3,3,4-trimethyldecane, 3,4,5,6-tetramethyloctane and butylated hydroxy toluene) were detected from *P. chrysogenum*, which was found to be the most attractive fungus. Among them, 1,5-octadien-3-ol and 2,6,10-trimethyldodecane were only produced by *P. chrysogenum*. The compounds common to *C. cladosporioides* or *A. flavus*, which were the second-most attractive fungi, were styrene, 1-octen-3-ol, 4-methylundecane, 3,4,5,6-tetramethyloctane and butylated hydroxy toluene. The compounds common to *A. tubingensis*, which was the least attractive fungus, were 3-octanone and butylated hydroxy toluene.

4. Discussion

The most common fungi in food factories and pharmaceutical factories in Japan are reported to be *Aspergillus*, *Penicillium*, and *Cladosporium* (Takatori, 2004; Morozumi, 2008; Tani and Ito, 2006). In our previous study, these fungi were found on indoor floors and among homogenized minute brown scavenger beetles (Latriidae) captured in a building in Narashino, Chiba Japan (Yoshinami et al., 2018). Thus, in the present study, four species (*A. tubingensis*, *A. flavus*, *P. chrysogenum*, *C. cladosporioides*) found among collected fungi were considered suitable for evaluating the fungal feeding preferences of *L. bostrychophila*.

The minimum water activity needed for the growth of the four fungi has been reported to be 0.77 for *A. tubingensis*, 0.84 for *A. flavus*, 0.78 for *P. chrysogenum* and 0.86 for *C. cladosporioides* (Corry, 1987). These fungi are not able to grow under conditions of humidity control. However, in an environment where the temperature and humidity change drastically, dew condensation is likely to occur on the walls and ceilings of facilities, air conditioning equipment, etc., and there are many spots where the local humidity is actually quite high. Therefore, even though these fungi cannot grow under conditions of low water activity, the fungi and associated insects can still grow and spread in food factories and food storage facilities where the humidity is controlled. We focused on the most common fungi found in the food industry in Japan in order to examine the fungi preferences of *L. bostrychophila*.

The 9d-PDC test showed that, among the fungus species tested in this study, *P. chrysogenum* was the most attractive fungus for *L. bostrychophila* (Fig. 3), followed by *A. tubingensis* and *C. cladosporioides*. Mills et al. (1992) ranked 19 species of seed-borne fungi that were the most suitable for the feeding and multiplication of *L. bostrychophila*. In their ranking, *P. chrysogenum* was classified into the top group of multiplicable fungi among storage fungi. Our results correlated with their report. Furthermore, Sinha and Mills (1968) reported that *Tyrophagus putrescentiae* (Schrank), which is a pest that tends to proliferate in stored food, also preferred *P. chrysogenum* as feed. However, the data on the

Table 2
Chemical compounds detected as odorous substances in four fungus species.

Chemical compounds name	Fungus species			
	Aspergillus tubingensis	*Aspergillus flavus*	*Penicillium chrysogenum*	*Cladosporium cladosporioides*
styrene		●	●	●
1,5-octadien-3-ol			●	
1,7-octadien-3-ol		●		
2,5,5-trimethyl-2-hexene	●[a]	●		
1-octen-3-ol		●	●	
2-octen-1-ol	●	●		
3-octanone	●	●	●	
2,2-dimethyldecane				●
etyl caproate	●			
propionic acid	●			
5-ethyl-2,2,3-trimethylheptane	●			●
2,6,7-trimethyldecane				●
2-isopropyl-5-methyl-1-hexanol				●
4-methylundecane			●	●
2,3,6,7-tetramethyloctane	●			
2,6,10-trimethyldodecane			●	
3,3,4-trimethyldecane			●	●
3,4,5,6-tetramethyloctane			●	●
3,7-dimethylnonane				●
3-methylbutanoic acid, pentyl ester	●			
1,2,3,6-tetramethylbicyclo[2.2.2]octa-2,5-diene		●		
beta-elemene		●		
1-isopropyl-4-methyl-7-methylene-1,2,3,4,4a,5,6,7-octahydronaphthalene		●		
beta-cubebene		●		
2,6-di(tert-butyl)-4-hydroxy-4-methy l-2,5-cyclohexadien-1-one	●			
1,beta,4,beta,H,10,beta,H-guaia-5,11-diene		●		
2,6-bis(1,1-dimethylethyl)-4-methylene-2,5-cyclohexadien-1-one	●			
beta-selinene		●		
alpha-gurjunene		●		
butylated hydroxy toluene	●	●	●	●
1-isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6,8a-octahydronaphthalene		●		
delta-cadinene		●		
2-methylpropanoic acid, 1-(1,1-dimethylethyl)-2-methy l-1,3-propanediyl ester	●			
1,1,4,7-tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol		●		
1-isopropyl-4,7-dimethyl-1,3,4,5,6,8a-hexahydro-4a(2H)-naphthalenol		●		

[a] Symbol ● shows the chemical compound detected from each fungus.

factors that render *P. chrysogenum* attractive to these pests are lacking.

Our results suggested that the morphological characteristics of the fungi were one potential factor. Velvety fungi (200 μm tall with high density), such as *P. chrysogenum* and *C. cladosporioides*, were preferred as feed. The trophi of *L. bostrychophila* are located on the lower surface of the head, allowing the insects to easily eat food below their head (Fig. 5). In the present study, the colony height of *P. chrysogenum* and *C. cladosporioides* was about 200 μm, which were lower than the body height of *L. bostrychophila* (300–400 μm). The hyphae and spores of these fungi showed dense growth under the trophi of the insects, allowing *L. bostrychophila* to easily eat these fungi. Regarding *A. tubingensis*, to which the insects were drawn similarly to *C. cladosporioides*, most hyphae and spores remained on the paper disc (data not shown). These results suggested that the insects were drawn to the *A. tubingensis* disc for a reason other than to eat. One possibility is that the insects may consider *A. tubingensis* useful as a hiding place, since the hyphae are taller than their body height. In addition, *A. tubingensis* showed strong acidity (Table 1). Interestingly *A. niger*, which belongs to the same taxonomical group as *A. tubingensis*, is classified as having a low feeding attraction (Mills et al., 1992). Since *A. niger* is known to produce citric acid (Nielsen et al., 2009), the release of acidic metabolites from fungi might be related to the *L. bostrychophila* feeding behavior.

The luminescence of the fungi may also be related to the *L. bostrychophila* feeding behavior. Briscoe and Chittka (2001) reported that insects were most sensitive to UV light of 350–385 nm in wavelength. Diaz-Montano et al. (2016, 2018) reported that psocids strongly responded to wavelengths in the UV spectrum. They found that the response differed according to the psocid species and that 351-nm UV light was the most attractive for *L. bostrychophila*. We suspected that *L. bostrychophila* might recognize feeding substances in the UV spectrum. In our study, the two most preferred fungi showed no fluorescence. Therefore, the presence or absence of fluorescence might influence the *L. bostrychophila* feeding preference (Fig. 4).

It is well known that insects seek food using their well-developed olfactory sense and a remarkable capacity to sense a wide range of volatile chemicals in their environment with high sensitivity and specificity (Hansson, 1999; Breer et al., 2019). The odor of fungi must be an attractive factor with a high priority. In the HOD test, the number of insects attracted to *P. chrysogenum* was highest, which is the result as found with the 9d-PDC test (Fig. 6); this suggests that *P. chrysogenum* produced the most attractive odor for evoking feeding behavior.

Based on this finding, the odorants produced by *P. chrysogenum* were next compared to those produced by other fungi. According to the GC/MS analysis in this study, 35 odor compounds were detected from the 4 fungi species as major peaks. Nine of these odorants were detected from *P. chrysogenum*, of which 1,5-octadien-3-ol and 2,6,10-trimethyldodecan were detected only in *P. chrysogenum* (Table 2).

Vanhaelen et al. (1980) investigated *T. putrescentiae*, which is a type of mite that feeds on *Trichothecium roseum*. They found that *T. roseum* produced 1,5-octadien-3-ol at low concentrations and that the compound was a potent attractant for *T. putrescentiae*. This suggested that *L. bostrychophila* might recognize 1,5-octadien-3-ol as an attractive substance for evoking fungal feeding behavior, similar to *T. putrescentiae*. Davis et al. (2013) proposed semiochemical interactions between microorganisms and insects by way of microbial volatile organic compound production. They reported numerous instances of odorous substances from fungi being closely associated with insect behaviors. Based on these findings, these two odorants are considered to be attractive candidate odors for inducing feeding behavior of *L. bostrychophila*. Green (2005, 2008) showed that the attractiveness of the fungal extracts for *L. bostrychophila* was based on the concentration. Future studies should therefore examine the relationship between the concentration and feeding behavior in order to determine which compounds act as an attractive odor.

In conclusion, this study revealed that *P. chrysogenum* was the fungus most frequently selected by *L. bostrychophila* among four fungus species that are commonly observed in the food industry in Japan. We analyzed the factors attracting *L. bostrychophila* to these species to identify the common characteristics shared by the preferred fungi. *P. chrysogenum*, which was the fungus most preferred as feed, possessed a velvety surface, shorter colony height than the insects' trophi and no luminescence. In addition, these fungi produced specific odors, suggesting that the height, density and luminescence of the fungi may function as attractive factors for *L. bostrychophila*.

Two applications of the present findings are proposed. First is the early prediction of the risk of insect occurrence by monitoring the presence of preferred fungi for *L. bostrychophila* in food factories and food storage facilities. By actively removing the high-risk fungi that grow in these facilities, the infestation of these insects can also be prevented. Second, understanding the common characteristics revealed in the present study may aid in the development of tools, such as traps for monitoring and capturing insects, thereby leading to effective IPM control.

CRediT authorship contribution statement

Makoto Yoshinami: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Rikuya Machida:** Methodology, Investigation. **Naoki Kobayashi:** Writing - original draft, Writing - review & editing. **Yoshiko Sugita-Konishi:** Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing, Project administration. **Katsunori Furuhata:** Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jspr.2020.101659.

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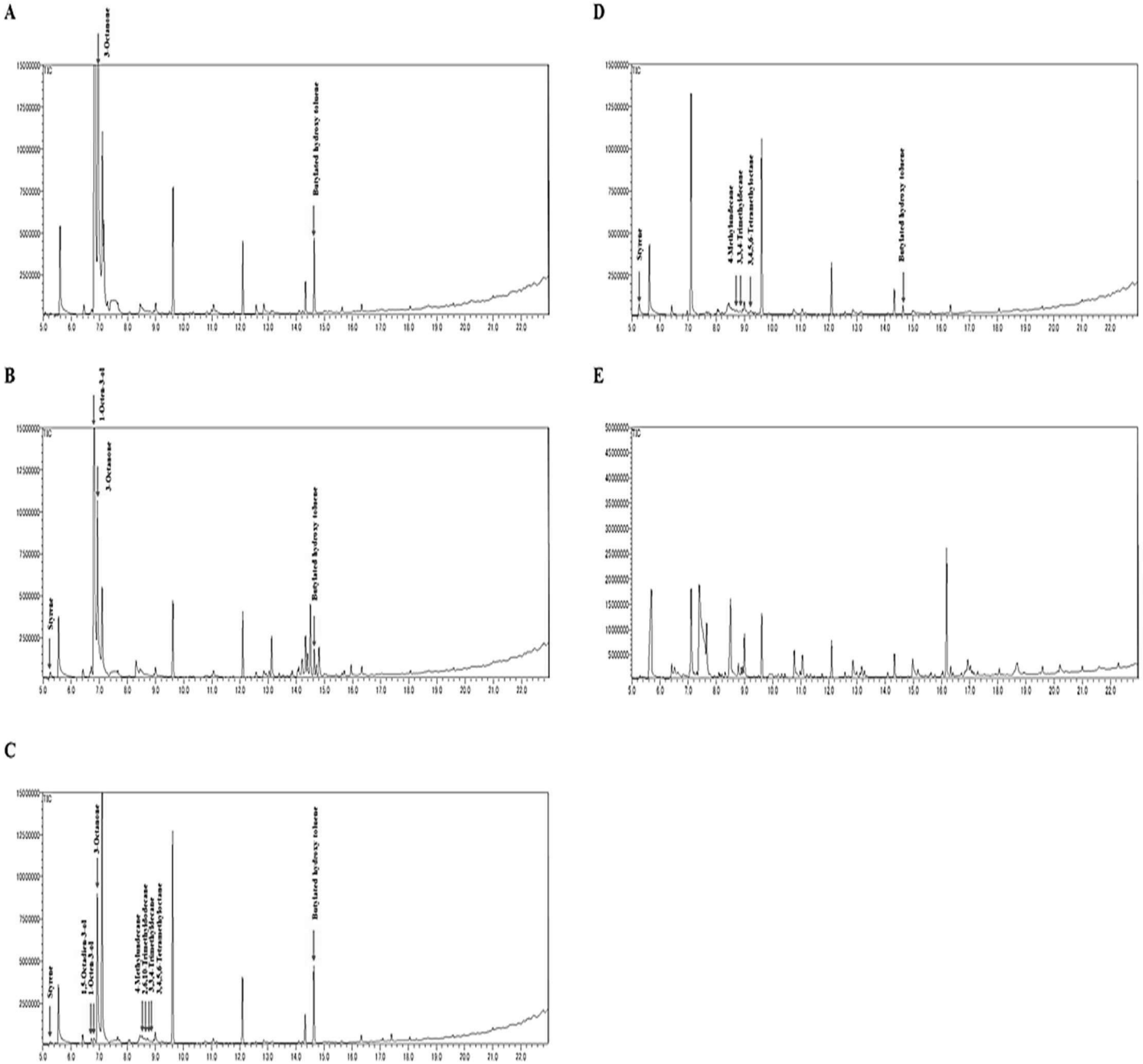

Fig. S1. GC chromatogram of the odorous fungal compounds in the fungus-cultured paper disc with each of the four fungus species, as determined by headspace SPME. A: *Aspergillus tubingensis*, B: *Aspergillus flavus*, C: *Penicillium chrysogenum*, D: *Cladosporium cladosporioide*, E: Control disc. Arrow symbol: Nine chemical compounds detected from *Penicillium chrysogenum*.

出　典

本論文は以下に公表した。

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