Influence of temperature on fruit body emergence of the basidiomycetes, Bovista dermoxantha, Vascellum curtisii and Conocybe lactea, on turf of bentgrass, Zoysiagrass and bluegrass in summer

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Summary: The relationship between temperature and fruit body emergence in summer in three fairy ring pathogens of basidiomycetes, Bovista dermoxantha, Vascellum curtisii and Conocybe lactea, on turf was examined using the method developed to predict the timing of emergence in pest insects. The number of fruit bodies at the turf study site in Chiba, Japan, was recorded together with average temperature at the weather station from 1999 to 2003. The lower theoretical developmental thresholds (the developmental zeros) for mycelial growth in B. dermoxantha, V. curtisii and C. lactea were estimated to be 14.6°C, 17.0°C and 8.8°C, respectively, based on the linear relationships observed in the temperature ranges 5-35°C. The cumulative effect of average daily temperatures that exceeded the developmental zeros required for each fungus (total effective temperature) was assessed. The probits of the percentage of total fruit bodies against the total that emerged over the five-year period of the study correlated with the total effective temperature to give a ratio of contribution exceeded near 0.6. Similar correlations were also found between probits obtained from daily recordings of fruit body numbers in the observation plots under 170 m² and total effective temperature. The observations of development in B. dermoxantha and C. lactea revealed that the average diameter and fruiting period of the fruit bodies was 11.5 mm and 5.8 days, and the average height and fruiting period were 36.8 mm and 1.8 days, respectively. One of the characteristics for these fungi is that they are short-lived.

Introduction

Environmental factors have been demonstrated to physically and chemically affect in vitro fruit body formation in basidiomycetes. In the field, a decrease in temperature and rainfall generally induce fruit body formation. However, the emergence of fruit bodies on turf in summer appears to be regulated by the cumulative effect of temperature and not by a decrease in temperature or rainfall. On the other hand, in studies of applied entomology, the development of insect pests was affected by cumulative temperatures, and a liner relationship was observed between the probability units transformed from percentages of cumulative adult emergence numbers and cumulative temperatures above lower developmental thresholds logically calculated (developmental zero). Here, we examined whether this method of analyzing insect populations could be applied to the relationship between temperature and fruit body emergence of the causal agents of fairy rings on turf, Bovista dermoxantha (Vittad.) de Toni (Fig. 1A), Vascellum curtisii (Berk.) Kreisel (Fig. 1B), and Conocybe lactea (J. Lange) Metrod (Fig. 1C) on turf. The former two are oval puffballs causing fairy rings on turfgrass and the latter one is mushroom-shaped, which only develops fruit bodies on turf but causes no damage on turfgrass.

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Materials and Methods

1. Mycelial growth of three fungi

Modified Czapek and Dox medium was used for isolation, maintenance, inoculum preparation and mycelial growth experiment of the three fungi, B. dermoxantha, V. curtisii and C. lactea. The medium contained 15 g sucrose, 5 g yeast extract (Difco, Detroit), 5 g polypepton (Nihon Pharm., Osaka), 1 g KH₂PO₄, 0.5 g MgSO₄•7H₂O, 0.5 g KCl and 0.01 g FeSO₄•7H₂O in 1 L distilled water. For isolation, the tissue inside a fruit body collected at the study site described below was cultured on the agar plate medium with 1.5% agar (w/v). For the mycelial growth experiments, mycelial pellets (4-mm diameter) from each fungus were transferred from the agar plate medium onto 10 ml of the medium in ten 100-ml Erlenmeyer flasks. These B. dermoxantha, V. curtisii and C. lactea cultures were then statically incubated at temperatures ranging from 5°C to 45°C at 5°C intervals for 7 days, 14 days and 12 days, respectively. Mycelia were collected using a nylon cloth and dried to constant weight at 95°C and weighed.

2. Field study

The study was conducted on a turfgrass study area in Chiba City, Chiba Prefecture. The turf consisted of bentgrass (Agrostis palustris Huds.; 1,860 m²), Zoysia-grass (Zoysia matrella Merr.; 5,675 m²) and bluegrass (Poa pratensis L.; 300 m²). The dates of emergence and numbers of fruit bodies for the three fungi were recorded from 1999 to 2003 at seven-day intervals. Fruit body numbers of the three fungi emerged at observation plots were also noted: B. dermoxantha appeared on bentgrass turf of 1 m² in 1996, 166 m² and 170 m² in 1998, and 170 m² in 1999 and on Zoysia-grass turf of 170 m² in 1999, V. curtisii appeared on bentgrass turf of 112 m² in 1999, and C. lactea appeared on Zoysia-grass turf of 170 m² in 1999. The average temperatures in 1996 and from 1998 to 2003 were obtained from meteorological records at Chiba Weather Station, 12 km distant from the study site.

Morphometric analyses were undertaken twice a day on the fruit bodies of B. dermoxantha and C. lactea from June to August 1999. These included measurement of the longer oval diameters of 33 B. dermoxantha fruit bodies and the stipe lengths (=heights) of six C. lactea fruit bodies in the other three 0.5 m quadrates newly constructed on bentgrass turf in the study area. Fruit bodies of B. dermoxantha and C. lactea were collected in the study area on August 2003. Longer and shorter diameters, and heights in these 30 fruit bodies of B. dermoxantha, and longer and shorter pileus diameters, and stipe lengths and diameters in 12 fruit bodies of C. lactea were measured. These were then weighed after drying as described above.

3. Calculations

The developmental zeros for the mycelial growth rates of the three fungi were calculated using linear regressions of the mycelial weight data obtained in the experiments described above. The increasing values of mycelial growth according to the increase of temperature were used for the calculation. Daily average temperatures above the developmental zero for each fungus were calculated by cumulatively adding daily average temperatures that exceeded the temperature requirements of development for single years. These temperatures, hereafter referred to as total effective temperature, provided by a day degree unit, were derived for 1999 to 2003. Percentages of cumulative fruit body numbers against the total were transformed to probability units, probits, by the probit
method\(^1\). Regressions of probits for the percentage of cumulative fruit body numbers for each fungus and total effective temperature were then performed against the data for the five years of the study, single years, and turfgrass-type, and the total effective temperatures required for 50% fruit body emergence calculated were then figured out.

Based on daily observation data of the fruit body numbers for the three fungi in the observation plots in 1996, 1998 and 1999, the regressions were also derived and total effective temperatures required for 50% fruit body emergence was calculated.

**Results**

1. Developmental zeros of three fungi induced from mycelial growth

Figure 2 shows the mycelial growth rates for each fungus plotted against temperature. The developmental zeros for mycelial growth in *B. dermoxantha*, *V. curtisii* and *C. lactea* were estimated to be 14.6°C, 17.0°C and 8.8°C based on the linear relationships observed in the temperature ranges 15–35°C, 20–30°C and 5–30°C, respectively.

2. Relationship between total effective temperatures and probits of cumulative fruit body numbers

Percentages of total fruit body numbers for each fungus in the five successive years plotted against the calendar dates (Fig. 3) revealed a non-linear relationship between both axes, based on the data through 1999–2000. The regression analyses using the probits for cumulative fruit body numbers against the total effective temperatures for the five years of the study revealed that fruit body emergence for each fungus could be applied to the following linear regressions (Fig. 4):

For *B. dermoxantha*, \[Y = 0.0055X + 1.0815,\]

for *V. curtisii*, \[Y = 0.0038X + 2.1964,\]

for *C. lactea*, \[Y = 0.0018X + 2.2454.\]

where \(Y\) is the probit for cumulative fruit body number as a percentage and \(X\) is the total effective temperature. \(Xs\) for \(Ys = 5\) imply total effective temperatures required for 50% cumulative fruit body emergence. Table 1 shows the regression equations for the three fungi considering the total duration of the study, individual years, turfgrass-type, ratios of contribution (\(R^2\)), and total effective temperatures required for 50% cumulative fruit body emergence. For *B. dermoxantha* and *C. lactea*, the ratio of contribution for the duration of the study was ranged from 0.91 to 0.97, but for *V. curtisii* it was ranged from 0.59 to 0.86.

In *B. dermoxantha* and *C. lactea*, the contribution ratios derived from the regression analysis using the probits for individual years revealed high; i.e. more than 0.9. On the other hand, the ratios for *V. curtisii* derived from the data for single years were around 0.6 with one exception. Among the turfgrass species, the highest ratio of contribution appeared in bentgrass turf in all fungi. No distinct tendency was apparent in comparisons in descending order of the total effective tempera-

![Fig. 2 Mycelial growth of *B. dermoxantha*, *V. curtisii* and *C. lactea* at different temperatures. The bold lines show the linear regression equations based on the indicated temperatures shown as solid circles for each fungus. The open circles were the mycelial growth rates that were not used for the linear regression equations.](image-url)

![Fig. 3 Relationship between cumulative fruit body numbers (%) of *B. dermoxantha*, *V. curtisii* and *C. lactea* and observation dates.](image-url)
Fig. 4 Relationship between total effective temperatures and probits of cumulative fruit body numbers (%) of B. dermoxantha, V. curtisii and C. lactea. The developmental zeros: B. dermoxantha; 14.6 °C; V. curtisii, 17.0 °C; C. lactea; 8.8 °C. The linear regression equations and ratios of contributions ($R^2$) are based on the total data from 1999 to 2003.

Fig. 5 Relationship between probits of cumulative fruit body numbers (%) of B. dermoxantha, V. curtisii and C. lactea observed daily in 1–170 m² turf and total effective temperatures. Developmental zeros are shown in Fig. 3. Nos. (1-7) are the same as those in Table 2; 1Bd,B, 1996: B. dermoxantha on 1 m² bentgrass turf in 1996, 2Bd,B, 1998: B. dermoxantha on 166 m² bentgrass turf in 1998, 3Bd,B, 1999: B. dermoxantha on 170 m² bentgrass turf in 1999, 4Bd,B, 1999: B. dermoxantha on 112 m² bentgrass turf in 1999, 5Bd,Z, 1999: B. dermoxantha on 170 m² Zoysiagrass turf in 1999, 6Vc,B, 1999: V. curtisii on 112 m² bentgrass turf in 1999, 7C1,Z, 1999: C. lactea on 170 m² Zoysiagrass turf in 1999.

Figures for 50% cumulative fruit body emergence in each year among the three fungi. Furthermore, the lowest total effective temperatures appeared in bentgrass turf among the three turfgrasses for all fungi.

The probits for total daily fruit body number in the three fungi in the observation plots are shown relative to total effective temperatures in 1996, 1998 and 1999 in Fig. 5. Based on those data, regression equations between the probits for percentages of cumulative fruit body numbers and total effective temperatures were figured out (Table 2). Although the actual effective temperatures for 50% cumulative fruit body emergence differed from the theoretical values in Table 2 for each year, the linear regressions showed contribution ratios above 0.7. This implies that the total effective temperatures could be explained by the probits of the percentages for the cumulative fruit body emergence in these fungi at levels exceeding 70%.

3. Daily development of B. dermoxantha and C. lactea

To assess the development of fruit bodies, oval fruit bodies of B. dermoxantha and mushroom-shaped fruit bodies of C. lactea were measured twice a day. In mature B. dermoxantha, the average longer diameter was 11.5 mm (maximum 27 mm, minimum 2 mm). Fruit bodies increased in diameter until they attained to their maximum size. Their surfaces then changed from white to brown before sporulation, senescence and death. The fruit bodies of 36% became reduced in diameter after maturity and the average fruiting period was 5.8 days (maximum 12 days, minimum 2 days). The average stipe length (height) of C. lactea was 36.8 mm (maximum 64 mm, minimum 15 mm) and the average duration of the fruiting period was 1.8 days (maximum 2 days, minimum 1 day).

The average longer diameter of B. dermoxantha fruit bodies was 10.3 mm, the average shorter diameter 8.5 mm and average height 9.1 mm, and the average weight 81.3 mg. The average longer diameter of C. lactea fruit bodies was 8.6 mm, the average shorter diameter of pilei 6.8 mm, the average stipe diameter 1.0 mm and average length 34.3 mm, and the average weight 22.2 mg.

Discussion

For the basidiomycete fungi, B. dermoxantha, V. curtisii and C. lactea, grown on turf, the emergence of fruit bodies was observed to correlate positively with total effective temperatures. This fact was identical with that a liner relationship was observed between the probits from percentages of cumulative adult emergence numbers and cumulative temperatures above lower developmental zero in applied entomology. B. dermoxantha and C. lactea especially showed the ratios of contribution near 0.9. For V. curtisii, the low
Table 1  Fruit body emergence of *B. dermoxantha*, *V. curtisii* and *C. lactea* on turfs of bentgrass, Zoysiagrass and bluegrass in total 7,835 m² study area observed weekly from 1999 to 2003

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Turfgrass</th>
<th>Year</th>
<th>No. fruit bodies</th>
<th>Regression equation</th>
<th>Ratio of contribution (R²)</th>
<th>Total effective temperature for 50% body emergence (day degree)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined</td>
<td>Total</td>
<td>1999</td>
<td>4,068</td>
<td>Y = 0.0046 X + 1.6493</td>
<td>0.9614</td>
<td>728</td>
</tr>
<tr>
<td>B. dermoxantha</td>
<td></td>
<td>2000</td>
<td>3,010</td>
<td>Y = 0.0052 X + 1.6211</td>
<td>0.9644</td>
<td>650</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2001</td>
<td>2,330</td>
<td>Y = 0.0059 X + 0.9501</td>
<td>0.9679</td>
<td>686</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2002</td>
<td>3,826</td>
<td>Y = 0.0073 X + 0.8626</td>
<td>0.9675</td>
<td>803</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2003</td>
<td>517</td>
<td>Y = 0.0083 X - 1.0171</td>
<td>0.9106</td>
<td>725</td>
</tr>
<tr>
<td>Bentgrass</td>
<td>Total</td>
<td>1999</td>
<td>1,198</td>
<td>Y = 0.0028 X + 2.3440</td>
<td>0.5918</td>
<td>949</td>
</tr>
<tr>
<td>Zoysiagrass</td>
<td>Total</td>
<td>2000</td>
<td>106</td>
<td>Y = 0.0028 X + 1.5129</td>
<td>0.6944</td>
<td>758</td>
</tr>
<tr>
<td>Bluegrass</td>
<td>Total</td>
<td>2001</td>
<td>704</td>
<td>Y = 0.0050 X + 0.8815</td>
<td>0.6139</td>
<td>824</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2002</td>
<td>977</td>
<td>Y = 0.0061 X + 0.9281</td>
<td>0.8606</td>
<td>668</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2003</td>
<td>1,346</td>
<td>Y = 0.0042 X + 2.7770</td>
<td>0.6561</td>
<td>529</td>
</tr>
</tbody>
</table>

Table 2  Fruit body emergence of *B. dermoxantha*, *V. curtisii* and *C. lactea* in observation plots on turfs (1–170 m²) of bentgrass, Zoysiagrass and bluegrass observed daily in 1996, 1998 and 1999.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Turfgrass</th>
<th>Area (m²)</th>
<th>Year</th>
<th>No. fruit bodies</th>
<th>Total effective temperature for actual 50% emergence (day degree)</th>
<th>Regression equation</th>
<th>Ratio of contribution (R²)</th>
<th>Total effective temperature for 50% fruit body emergence (day degree)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. dermoxantha</td>
<td>Bentgrass</td>
<td>1996</td>
<td>1</td>
<td>248</td>
<td>736</td>
<td>Y = 0.0057 X + 1.7590</td>
<td>0.7441</td>
<td>568</td>
</tr>
<tr>
<td>B. dermoxantha</td>
<td>Bentgrass</td>
<td>1998</td>
<td>166</td>
<td>508</td>
<td>501</td>
<td>Y = 0.0042 X + 2.5729</td>
<td>0.6954</td>
<td>578</td>
</tr>
<tr>
<td>B. dermoxantha</td>
<td>Bentgrass</td>
<td>1999</td>
<td>170</td>
<td>2,953</td>
<td>545</td>
<td>Y = 0.0063 X + 1.9781</td>
<td>0.8778</td>
<td>570</td>
</tr>
<tr>
<td>B. dermoxantha</td>
<td>Bentgrass</td>
<td>1999</td>
<td>112</td>
<td>8,187</td>
<td>839</td>
<td>Y = 0.0065 X - 0.5885</td>
<td>0.9614</td>
<td>860</td>
</tr>
<tr>
<td>B. dermoxantha</td>
<td>Zoysiagrass</td>
<td>1999</td>
<td>170</td>
<td>57</td>
<td>959</td>
<td>Y = 0.0127 X - 7.1664</td>
<td>0.8341</td>
<td>958</td>
</tr>
<tr>
<td>V. curtisii</td>
<td>Bentgrass</td>
<td>1999</td>
<td>112</td>
<td>103</td>
<td>935</td>
<td>Y = 0.0090 X - 4.1726</td>
<td>0.8608</td>
<td>927</td>
</tr>
<tr>
<td>C. lactea</td>
<td>Zoysiagrass</td>
<td>1999</td>
<td>170</td>
<td>605</td>
<td>1,611</td>
<td>Y = 0.0020 X + 1.8822</td>
<td>0.9487</td>
<td>1,550</td>
</tr>
</tbody>
</table>

Y: The probit of the percentage of cumulative fruit body numbers, X: The total effective temperature. Dashess indicate not surveyed.
value of ratio of contribution, near 0.6, implies that the fruit body emergences were controlled by unknown factors. A positive correlation was observed between the probit, across and within years, and the percentage of fruit body emergence in the observation plots. In a field study, an ectomycorrhizal fungus, *Tricholoma bakamatumake* Hongo, showed temperature-affected fruit body development after fruit body initiation. In another ectomycorrhizal fungus, *Tricholoma matsu-take* (S. Ito & Imai) Sing., temperature was also demonstrated to affect primordial formation and fruit body development. For woodland fungi, temperature and soil moisture, which is influenced by amount of rainfall, were found to influence fruit body emergence. Similarly, *in vitro* fruit body emergence of mushroom-forming fungi has been demonstrated to be affected by physical and chemical factors, such as humidity, temperature, light, carbon dioxide, nutritional factors and metabolic bases.

In 1999, days on which the average temperature firstly exceeded the developmental zero for *B. dermoxantha*, *V. curtisii* and *C. lactea* were March 15, April 2 and January 20, respectively. This would imply that an increase in the growth of vegetative subsurface-mycelia occurred on the aforementioned dates in preparation for emergence of the fruit bodies. Here, the mycelial growth rates of *B. dermoxantha* and *C. lactea* were 3.5 mg at 20°C and 0.2 mg at 15°C per day, respectively. Therefore, to produce a 81.3 mg of *B. dermoxantha* fruit body measuring 10.3×8.5×9.1 mm could be expected to take 23 days at under 20°C, and a 22.2 mg of *C. lactea* pileus that measured 8.6×6.8 mm with a stipe of 34.3 mm could be expected to take 111 days at under 15°C. These temperatures were similar to the average monthly temperatures obtained from April to July (20.5°C) in 1999, and from January to August (16.5°C); as the regression analyses showed that July and August were the months estimated as the emergence periods of 50% fruit bodies of *B. dermoxantha* and *C. lactea* based on the theoretical developmental zero, respectively.

One of the developmental characteristics for both *B. dermoxantha* and *C. lactea* is that they are short-lived, dying within 5.8 and 1.8 days, respectively. The limited longevity of fruit bodies makes it difficult to control fairy ring diseases, because the location of mycelia under the soil surface can only be inferred from the location of the fruit bodies. However, fruit bodies above soil surface are expression of mycelia in soil, and it is the mycelia of pathogenic fungi that need to be destroyed in order to combat such an infestation. These turfgrass fungi are particularly problematic for the managers of golf courses as the two puffball fungi, *B. dermoxantha* and *V. curtisii*, are pathogenic on turfgrass. Moreover, the fruit bodies of the three fungi can alter the trajectories of golf balls moving over turf and be eyesore in course environment. This study is new to describe the fruit body development of fairy ring fungi in the field, since few studies have focused on the daily development of fairy ring fruit bodies so far.

**References**


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