Impact of Packaging on Mold Growth on an Orange

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Problem Statement

Exploring the realm of the fungal kingdom presents a considerable challenge in comprehending our planet's biosphere. How can we enhance our understanding of mold, a primary contender for food resources, and leverage this knowledge to minimize decay and waste through optimized packaging strategies? Motivated by the contemporary necessity to mitigate ecological impact, we aim to investigate the intricacies of fungal growth, a process mainly influenced by temperature and humidity, which are highly affected by the storage environment and the packaging type. Based on these features, we have sought to design a multi-agent model that strikes the right balance between realism and simplicity in codifying mold growth behavior. Through this model, we hope to contribute to the advancement of sustainable resource management practices.

In our exploration, we have focused our attention on the specific scenario of a molding orange. The orange, in tandem with our model incorporating the three-dimensional aspects of NetLogo3D, serves as a tangible representation of perishable goods susceptible to mold growth. Thus, we aim to address the research question: "What is the effect of packaging shape and material in simulated fridge vs. no fridge environments on mold growth in a multi-agent-based model of an orange?" To this end, we have defined the following sub-questions:

1. What is the effect of packaging shape in simulated fridge environments on mold growth in a multi-agent-based model of an orange?
2. What is the effect of packaging material in simulated fridge environments on mold growth in a multi-agent-based model of an orange?
3. What is the effect of packaging shape in simulated room environments on mold growth in a multi-agent-based model of an orange?
4. What is the effect of packaging material in simulated room environments on mold growth in a multi-agent-based model of an orange?

By addressing these research questions, our multi-agent system aims to provide insights into the dynamic relationship between environmental elements, packaging strategies, and the growth patterns of mold on perishable items. Through this exploration, we strive to contribute to the broader understanding of fungal behavior and its implications for sustainable resource management.

Model Description and Methods

The model we implemented represents mold growth on an orange in different conditions. During the setup, patches that represent the skin and flesh of the orange, as well as air, are identified. In addition, if a packaging shape is selected (a cube or cuboid) as well as a packaging material (plastic or paper), the packaging configuration is modeled around the orange. (See Figure 1).

Multiple agents interact during a simulation: droplets of water appear randomly in air patches and move based on a Diffusion-Limited Aggregation model (Witten & Sander, 1981), used to capture the macro-behavior of particles depositing upon a surface while avoiding the complexity computational fluid dynamics introduces in accurately modeling air flow. If a humidity droplet encounters an orange skin patch, it dies and increases the patch's humidity; if it wanders too far away from the orange it stops being relevant and dies. The number of humidity droplets present at all times remains constant.

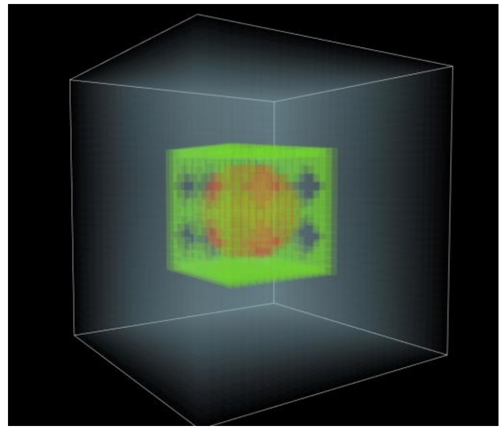


Figure 1: Simulated orange in cube packaging in NetLogo3D.

Our model assumes that microscopic spores are present homogeneously on the orange's surface, and so we have modeled significant mold germination and consequent visibility, rather than true microscopic spore counts. Thus, at every tick (approximately equal to 30 minutes), 5 nuclei try to sprout in random locations on the orange skin. Nuclei are agents that represent the fruiting bodies of mold, which are responsible for the spreading of spores. Their probability of germinating on the orange is calculated as follows: humidity of patch > (100 - spore-stickiness) / 2. Since spore-stickiness is a fixed value in our experiment set to 50, if the humidity of the patch is lower than 25, the nucleus dies. We have multiple fixed parameters in our model, chosen to simulate real-world conditions (Yackel et al., 1971; Dagnas & Membré, 2013), as shown in Table 1.

Once a nucleus sprouts, it will increase the probability of other nuclei sprouting around itself, modeling the fruiting body increasing the number of spores present in the air around it. The probability of achieving this is dependent on the 'spread speed' slider and the temperature. It is calculated

Table 1: Fixed parameters

Parameter name	Default value
orange-size	10
max_skin_humidity	50
spread_speed	35
spore-stickiness	50
num_hyphae	24
max_depth	4
holes	True

as follows: spread_speed * (0.5 + growth-temp / 3) > (random 5000). When this condition is fulfilled, the function that makes a nucleus sprout on that patch is called again.

Mold also penetrates the fruit's interior: 24 hypha agents are created from each nucleus in random directions. A hypha agent which lands in an air patch dies. A hypha agent which lands in an orange patch stays, enabling its spread within the fruit (See Figure 2). Hyphae are then represented by a chain of hypha agents, as at each tick a hypha agent lengthens the existing chain with a probability dependent on the spread_speed and temperature parameters according to the following formula: random 1000 < spread_speed * (0.5 + growth-temp / 3). Moreover, we have set the maximum length a chain of hypha agents can become, simulating hyphae behavior in a real-world scenario. At each time step, the hyphae chain also has a 25% chance of bifurcating, adding two new agents instead of one, pointing in two different directions. The maximum total chain length remains unchanged, and counting continues for the bifurcated hypha agent chain, disallowing infinite growth.

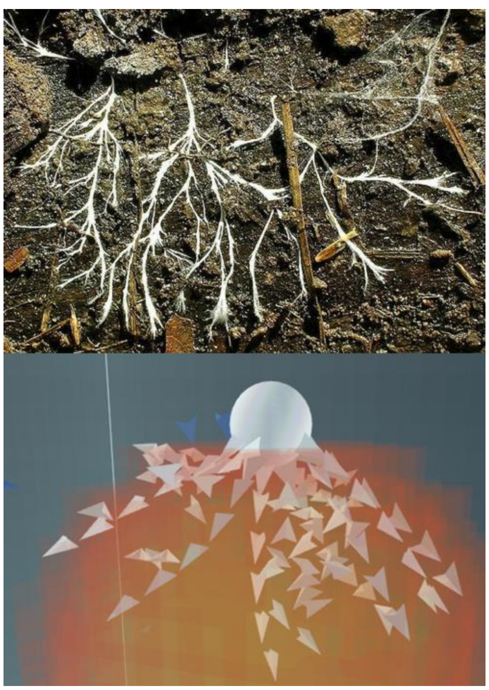


Figure 2: Real hyphae and simulated hyphae in NetLogo3D.

Lastly, we have properties linked to the material of the packaging and temperature. Humidity droplets will "bounce off" when coming in contact with packaging, mirroring their direction angle relative to the surface. Paper packaging also has a 10% chance of absorption of humidity upon contact (the humidity particle dying).

Temperature influences humidity, namely the number of droplets in the air according to the following formula: count drops < 5 + (10 + (2 * temperature / 3)) * (air-humidity / 100). It also impacts growth rate as *Penicillium digitatum* (green rot) typically grows rapidly at 20–25 °C but very slowly below 5 °C or above 30 °C (Hocking, 2014). We have modeled our system to behave similarly, using a linear decrease in mold growth as we move away from 25 °C. When temperature is set below 15 °C or above 35 °C, the growth rate is set to a constant 0.5 (Yackel et al., 1971; Dagnas & Membré, 2013).

As demonstrated, our implementation tries to mimic the phenomenon studied as much as possible while remaining relatively simple and providing relevant insights.

Methods

To collect our data, we used BehaviorSpace to run our simulation. The main outcome measured in our simulations is the percentage of orange patches that include at least one hypha. To study the effect of different parameters on it 40 runs for every condition were simulated. The parameters for each condition are shown in Table 2.

Table 2: Condition parameters.

Parameter name	'Fridge'	'Outside'
Humidity	25	50, 75
Temperature	5	20, 35
Packaging shape	None, cube, cuboid	None, cube, cuboid
Packaging material	Plastic, paper	Plastic, paper

During our simulated runs, we had the model stop when reaching 300 ticks or 200,000 mold agents. The reason for this is two-fold: 1) computational limitations, and 2) the observation that, in the simulated room environment, meeting either of these conditions resulted in approximately 30% of the orange being filled with mold. Considering that hyphae can only grow to a fixed depth, we believe this is enough to call the orange rotten.

Having collected our data, we wished to appropriately quantify the dynamics of mold growth in our model. Based on previous studies on mold spore germination (Dantigny et al., 2002), mold on organic surfaces (Dagnas et al., 2002; Berger et al., 2018), and exponential organic growth in general (Zwietering et al., 1990), we used a logistic model. An exponential growth model follows the equation:

$$(dN/dt) = bN$$

Where b > 0 and represents the rate of growth or the steepness of the curve. For logistic growth, bN is multiplied by a saturation term $(1 - N/d)$ to reflect resource limitation. Here d represents the saturation value for mold percentage where the rate of growth becomes zero. This is because eventually there will be no surface for the mold to grow on. Since this mathematically corresponds to the Verhulst equation, we have chosen it to represent mold growth (Bacaër, 2011):

$$dN/dt = bN(1 - N/d)$$

Solving the differential equation gives us:

$$N(t) = d/(1 + exp(-b(t - e)))$$

Where e is due to the constant of integration.
To fit the model data in R, we use the 'drc' package (Ritz et al., 2015) in which the sign of b is reversed, so the new model we use for the fit becomes:

$$N(t) = d/(1 + exp(b(t - e)))$$

To summarize the model parameters, e denotes the starting point for mold growth, b indicates the steepness of the growth curve and d represents the saturation value which accounts for the asymptotic saturation behavior associated with limited resource systems (Bacaër, 2011).

Having found our equation, we then proceeded to process our data in R. In accordance with our research question, our null hypothesis states that neither packaging shape, nor packaging material impact mold growth on an orange in our multi-agent-based model. We tested this by running a logistic model in R in which we used timesteps as predictors of mold percentage on the orange for each packaging shape and material condition.

To assess the difference between the 'Fridge' and 'Outside' conditions we ran a paired samples t-test to compare the means across conditions (packaging shape and material) at each timestep.

Results

Our model coherently displays the temporal behavior discussed above. Assuming that 50 ticks model one day of real time, it is clear that in a non-packaging condition, at 20-35 °C, mold sprouts consistently in proximity of the first day, in line with Yackel et al. (1971) and Dagnas & Membré (2013). On the other hand, packaging significantly impacts the initial event, delaying it to the second day in most cases. For the fridge simulated environment (5 °C), mold sprouts with the same timing, even though the growth curve is much less steep, as expected based on the experiments conducted by Gougouli & Koutsoumanis (2012).
All our five packaging conditions in both environmental conditions (fridge and outside) showed significant effects on all three logistic model parameters ($p < .001$) meaning they are able to significantly explain mold percentage values. The estimates for the model parameters are given in Table 3 and Table 4. Additionally, results with fitted curves are shown in Figure 3 and Figure 4.

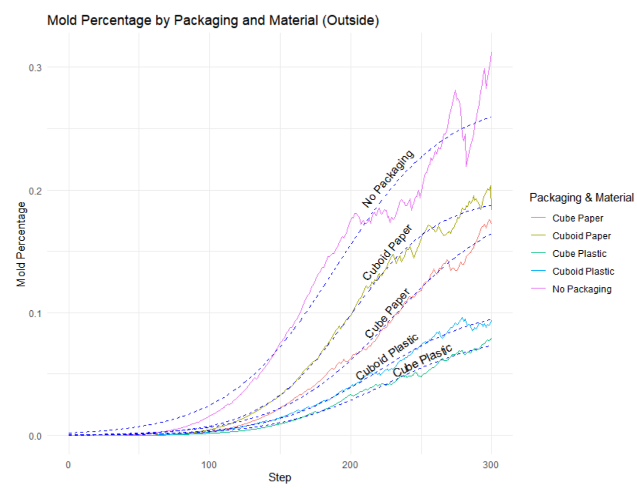
'Outside' Condition

Based on the d values, no packaging condition had the highest growth (d = 0.27). Cuboid shape had more mold growth than Cube shape for both Paper and Plastic. Paper material had higher mold growth than Plastic for both Cube and Cuboid packaging shapes.

Table 3: Summary of the logistic curve fitting for 'Outside' environment; df = 1490

Conditions	Parameter	Estimate
	b	-0.025
No Packaging	d	0.274
	e	189.71
	b	-0.025
Cube Paper	d	0.191
	e	229.24
	b	-0.025
Cube Plastic	d	0.083
	e	225.10
	b	-0.032
Cuboid Paper	d	0.194
	e	199.48
	b	-0.026
Cuboid Plastic	d	0.105
	e	219.96

Figure 3: Graphed data of the 'Outside' condition with fitted curve.



'Fridge' Condition

Based on the d values, no packaging condition again had the highest growth (d = 0.0614). Cuboid shape had higher mold growth than Cube shape for both Paper and Plastic. Paper material had higher mold growth than Plastic for each shape.

Condition Comparison

The Paired t-test testing the difference between mold percentage in 'Outside' condition and mold percentage in 'Fridge' condition across timesteps (ticks) suggests that the effect is positive, statistically significant, and large (mean difference = 0.05, 95% CI [0.04, 0.05], $t(300) = 17.03$, $p < .001$; Cohen's $d = 0.98$, 95% CI [0.84, 1.12])

Discussion

This study aimed to explore the influence of environmental factors and packaging strategies on mold growth in an agent-based model of a molding orange. Application of the model to both 'Outside' and 'Fridge' conditions, considering various packaging scenarios, yielded significant findings.

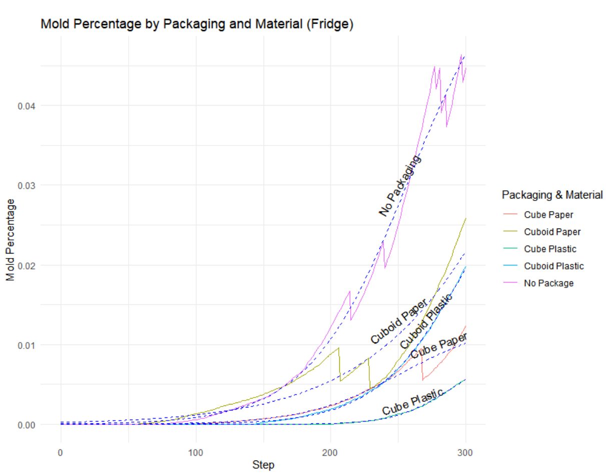
The logistic model, derived from previous studies on mold spore germination and exponential organic growth, effectively captured growth dynamics (Dagnas & Membré, 2013; Yackel et al., 1971). All five packaging conditions exhibited significant effects on model parameters, emphasizing the roles of packaging material and shape, regardless of air humidity and temperature.

Findings align with existing knowledge, providing a realistic representation within the simulated context. Temperature functioned with an optimum around 25° C, decreasing mold-growth when either higher or lower, in line with real-world scenarios (Dagnas & Membré, 2013; Yackel et al., 1971). Likewise, relative air-humidity levels are positively associated with mold-growth, as expected (Smilanick & Mansour, 2007). Finally, variation in package materials, as well as shapes, resulted in the expected outcomes, with tighter packaging decreasing mold growth, and plastic winning out over paper (Sicari et al., 2017; Anoraga & Bintoro, 2020).

Table 4: Summary of the logistic curve fitting for ‘Fridge’ environment; df = 1490

Conditions	Parameter	Estimate
No Packaging	b	-0.026
	d	0.061
	e	258.11
Cube Paper	b	-0.028
	d	0.012
	e	252.10
Cube Plastic	b	-0.049
	d	0.008
	e	287.73
Cuboid Paper	b	-0.015
	d	0.098
	e	380.31
Cuboid Plastic	b	-0.030
	d	0.037
	e	297.43

Figure 4: Graphed data of the ‘Fridge’ condition with fitted curve.



However, our model employs several composite features. For instance, the abstraction of air humidity in the model differs from real-world complexities, potentially causing breakdowns in extreme circumstances (Witten & Sander, 1981). Additionally, simulated humidity patches currently have an absolute threshold, whereas real-world humidity loss is likely a continuous process (Sanaeinejad, 2011). Our model also mirrors the way hyphae work in real life: spores germinate into hyphae and only then those connect to each other and grow to the point of developing a fruiting body.

Furthermore, physics components, including gravitational pull on the orange and stress between orange particles in order to simulate the orange collapsing, were considered but not included due to software and hardware constraints. For the same reason, we did not include the orange releasing its water into the environment upon injury, nor its usual shrinking behavior due to loss of water. Future research could explore more computationally intensive models using alternative software and increased computing power.

Finally, empirical validation against real-world scenarios could enhance the model's robustness, allowing us to refine it for more extreme cases, and exploring diverse scenarios with other fruits, varied food items, and different fungi would broaden the model's scope.

Conclusion

In conclusion, our agent-based model successfully simulated mold growth dynamics, offering insights into the impact of packaging and environmental conditions. Findings align with existing knowledge, highlighting the model's realism. However, much can be done to improve its realism. Refinement, validation, and exploration of new or unexplored features will contribute to its accuracy, facilitating nuanced investigations into mold dynamics and packaging strategies.

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Appendix

Appendix A: NetLogo3D code

With verbal permission from Dr. Wiltshire our NetLogo3D code is not included here as we wrote most of it ourselves. We took inspiration from the 3D DLA model (DLA3D) to model the movement of Humidity particles through the air. Earlier editions of our code also used code from the 2D Slime Mold Network (Slime Mold Network), but in later iterations this code was replaced by our own version. As agreed, comments about this are included in our NetLogo3D file to clarify where we took inspiration from.