The evolutionary impact of monoculture on crop pest resistance

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NS51: Research Design

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Questions about your study design	**Your response**
What is your research question?	What is the evolutionary impact of monoculture on radishes' pest resistance?
Write two hypotheses and put the one that you will focus on in your paper in bold.	**H1: The lack of genetic diversity in monoculture leads to the evolution of better-infected pests, thus worse pest resistance than polyculture.** H2: In monoculture, because radishes that can resist pests or diseases are more likely to survive and pass those genes onto their offspring, the radish population will develop better pest resistance.
For your bolded hypothesis that you will focus on in your paper, write three predictions. Choose one of those predictions to focus on in your paper and put it in bold. Your study design should propose how to test this prediction to determine support for or against your bolded hypothesis.	**P1: If similar seeds of radish are grown in monoculture and polyculture in similar conditions, the latter will develop fewer signs of pest damage.** P2: If similar seeds of radish are grown in monoculture and polyculture in similar conditions, the latter will have more genetic diversity. P3: If radish is moved from monoculture to polyculture, it will be more susceptible to pests and diseases.
What are the potential ethical considerations of your study?	1. The development of pests and diseases must pose no harmful impacts on human health/ the environment 2. Not leave a bad long-lasting impact on the land after experimenting so that the farmers can continue farming 3. Ensure participating farmers sustain their lives when

	giving their fields away for experiments
What type of research design will you use? [interventional study (e.g., RCT, quasi-experiment) or observational study (e.g., case-control study, cross-sectional study)]	Interventional study (randomized controlled trial) for 15 months
What sampling strategy will you use?	Stratified sampling based on soil type and agriculture technique (disproportionate sampling). Then, a random sample of fields is selected from each stratum to ensure the representation of different conditions. Desired total sample size is 700 fields
What are your treatment(s) [if applicable]	Intercropping radish with lettuce and spinach. Following established intercropping agriculture techniques.
What are your independent and dependent variables?	Independent variable: monoculture or polyculture Dependent variable: the incidence and severity of pest and disease damage through visual symptoms such as lesions, spots, wilting, stunting, or necrosis
What are your controls? [address group(s) and variable(s) as appropriate]	Monoculture group: Growing radish seed alone
What are potential confounding variable(s)?	1. Locations affect weather conditions and agricultural techniques 2. Soil type affects pest control. 3. Environmental factors: Temperature, humidity, sunlight, and precipitation.

	4. Seed quality: low-quality seeds may have lower germination rates, weaker and less healthy seedlings, and reduced genetic resistance to pests and diseases 5. Planting density: Usually the denser plants are grown together, the more probability of pests and diseases 6. Agricultural knowledge of farmers affects pest damage.
Does your study design properly address ethical considerations? [It's good to think of this at multiple points in the process, especially before collecting data]	1. Obtain informed consent from all participating farmers, and provide them with information about the potential risks and benefits of the study. 2. Follow strict agriculture regulations, monitor the effects of treatments on the local ecosystem and human health 3. Compensate participating farmers for any loss of income or inconvenience during the study period. 4. Re-train farmers after experimenting.

Introduction

Current agriculture, which is dominated by monoculture (single-species crop) is often compared to polyculture (intercropping various companion plants). Since monoculture crops are genetically identical, a pest or disease is more likely to infect the whole field than in polyculture crops where multiple barriers are available to prevent disease spread. Over time, better-infected pests survive and pass on to later generations in monoculture fields (Kremen & Miles, 2012).

The research question of this study is: What is the evolutionary impact of monoculture on radishes' pest resistance? I choose radish because its short life cycle (30 days) helps observe the evolutionary changes. My hypothesis is: The lack of genetic diversity in monoculture crops leads to the evolution of better-infected pests, thus worse pest resistance than polyculture crops. [1]

I leave two intermediary nodes of the mechanism as assumptions due to large supporting scientific evidence: [2]

1. Monoculture decreases genetic diversity: maize crop in Mexico (Atchison et al., 2016), soybean across Chinese, Japan, North America, and Brazilian (Carter et al., 2016).
2. Decreased genetic diversity increases vulnerability to pests and diseases (Gilbert, 2002), (Zhu et al., 2000), etc

To test this hypothesis, I propose a prediction that if similar seeds of radish are grown in monoculture and polyculture (independent variable) under similar conditions, the latter will develop fewer symptoms of pest damage (dependent variable).

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[1] #hypothesis development: I identify an alternative hypothesis to the chosen hypothesis in the cover sheet. For the chosen hypothesis, I provide the mechanism backed up by evidence (the evolution of better-infected pests), and the outcome (worse pest resistance than polyculture). I include comparison groups in my hypothesis in which monoculture crops act as a baseline for evaluating the pest resistance of poyculture.

[2] #Plausability: I identify 2 main assumptions in the hypothesis. I explained why the genetic diversity variable is not included in my research design to avoid over-complexity because it is highly plausible with a large amount of scientific evidence. The evidence also includes empirical field experiments.

Research design

I. Objectives

There have been insufficient interventional studies to establish causality between polyculture and pest resistance. Moreover, most of the interventional studies sample experimental fields without considering whether their history of agricultural techniques has been monoculture or polyculture. Researchers found that monoculture-based lands are prone to soil erosion, depletion of nutrients and natural microorganisms, which leads to a biased development of monoculture plants that have already adapted to these specific depletions (Zhu et al., 2000). Therefore, this study aims to propose a more comprehensive control of confounding variables to best mitigate the selection bias.

II. Location and duration

China is chosen for its familiarity with radish growth and collectivist agriculture where farmers share similar farming practices. The interventional study will be conducted in ten contiguous townships that participated in an interventional study in 1999 (Zhu et al., 2000): Chenguang, Dongba, Mianding, Nanzhuang and Xizhuang in Jianshui County; and Baxing, Baoxiu, Songchun, Maohe and Yafanzi in Shiping County. Those townships are close in weather conditions so that seasonal/ climate changes are reflected on all subjects. They are also acquainted with both monoculture and polyculture practices to avoid sample bias. [3]

The experimenting duration is 15 months consisting of 15 generations of radishes. This allows the effect of the natural selection of the best-infected pest genome in monoculture fields.

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[3] # evidence-based: I based my location selection on a previous scientific paper in the field. It helps me justify why I use China in general and 10 townships in specific. I explained the advantage of my location selection based on the evidence of the characteristics of those townships and link those advantages to the ability to minimize biases in my research design.

III. Comparison groups

There are 2 comparison groups:

- A genetic diversity treatment group (polyculture): Growing radish, lettuce, and spinach together. Those are the two most common companion plants with radishes.
- A control group with no genetic diversity manipulation (monoculture). Radish is grown alone.

As opposed to former interventional studies (Zhu et al., 2000), I do not include the third comparison group of higher-diversity polyculture (6 companion plants). This is because there could be a case when high-diversity fields have higher pest damage than low-diversity fields. I in turn could not falsify the prediction because one could attribute that result to the failure in design and control of too many companion plants at a time, not the effectiveness of polyculture in general. Therefore, the treatment group includes only the companion combination with strong historical and scientific evidence and protocols. This ensures any possible decrease/ insignificant increase in pest resistance of polyculture in comparison to monoculture could trustworthily falsify the prediction. [4] [5]

IV. Sampling method

1. *Population sampling*

First, researchers create a full frame of information about all fields (experimental units), eliminating those unqualified for growing radish to reduce noise in the result. Then, researchers

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[4] # testability: This paragraph explains why I exclude a third comparison group to ensure the result can falsify the prediction. This is what makes a prediction testable. Moreover, both the independent and dependent variables can be defined from the prediction (introduction part). The measurement for the dependent variable is provided, with 2 different symptoms to minimize bias and maximize accuracy in results (Interventional delivery part).

[5] #comparison group: The number of comparison groups and the purpose of each is justified and linked to #testability. I ensured that the chosen comparison groups can support/ falsify that the independent variable of interest is having a measurable effect (or not) on the dependent variable. What both groups will receive was listed with clear instructions.

split into 6 mutually exclusive stratum based on soil type (Sandy, clay, loam soil) and agriculture technique (Monoculture or polyculture). Each field fits into one stratum only to avoid an unequal probability of being sampled. Following disproportionate sampling, the sample size from each stratum is proportional to its size in population, with the same number of units selected from each (by simple random sampling). This is because I anticipate the number of polyculture fields will be fewer than that of monoculture, as well as variability in different soil types. Overall, this method ensures the best representation of the population while minimizing overrepresented or underrepresented sample distribution. The desired total sample size is 700 fields to maximize accuracy and account for outliers and dropout cases.

2. *Randomization*

Each field is randomly allocated into treatment and control groups to ensure the confounding variables are equally distributed across treatment and control groups. This ensures the observed result is due to the impact of monoculture and polyculture on pest resistance, not due to chances or third factors.

3. *Sampling to measure*

70% of radish plants in each field will be inspected with pest damage symptoms following simple random sampling (random number-generated selection process). This ensures the representation of fields' pest damage while saving time and resources. [6]

[6] # sampling: I justified the need for a method that leads to the most representative of the population through the objective statement. This explains why I choose stratified sampling. The location, duration, and process were also stated and estimated with numbers. A potential threat to bias was identified and resolved. I included holistically 3 sampling stages including sampling to measure.

V. Confounding variables

Researchers must ensure that treatment and control groups receive constant, similar and best growing conditions.

Before experimenting, researchers bring all the soil in both groups to the best growing condition for radish (well-drained soil with a pH between 6.0-7.0). Researchers use the highest-quality seeds to avoid pest outbreaks.

Both groups would follow a 10cm spacing between each plant and a 15cm spacing between each row. To avoid between-group contamination, each field will be physically separated and receive scheduled removal of infected plants to block transmission. Pesticides and fertilizers will not be used to ensure the natural development of plants. Moreover, farmers will receive training on either polyculture or monoculture, regardless of their prior knowledge about each technique.

VI. Interventional delivery

First, to mitigate performance and confirmation bias, researchers must get trained and monitored to adhere to a pre-defined standardized protocol. Double-blinding is not possible because farmers will follow either monoculture/ polyculture techniques and researchers can easily distinguish. After sampling, researchers must obtain informed consent and risk-benefit acknowledgment from all participating farmers. To avoid attrition bias of unequal dropout rate, researchers ensure stronger commitment agreement of treatment group due to more complex farming technique. On a weekly basis, each field will be visually inspected for the spread and severity of the two most common radish diseases: clubroot (number of galls on the roots) and black rot (number of yellowing and wilting leaves, and blackening veins and roots) (Babar et al., 2021). Researchers will use standardized measurement and encode processes (0 - no damage to 5 - severe damage) across both groups to minimize assessment bias. [7]

To address ethical considerations, researchers must monitor the effects of treatments on the local ecosystem and human health, such as soil conservation, erosion control, chemical reports, and replanting of native vegetation post-experiment if necessary. Researchers must also compensate participating farmers for any loss of income or inconvenience during the study period. After ending the experiment, researchers must train farmers again on any adaptive agriculture practices. [8]

Expected results and interpretations

Based on the prediction, the polyculture fields have fewer pest damage symptoms than the monocultures. Under rigorous control of confounding variables, this could suggest that polyculture aids pest and disease resistance without relying on pesticides and fertilizers. However, the generability is limited by (1) the specific location, (2) the specific combination of radish's companion plants, (3) the chosen agricultural protocol, (4) a timeframe of 15 months, which may not capture the full effects of intercropping and disease development. Further

[7] # Bias mitigation: I addressed performance bias (through training and protocols, prevention of between-group comtamination, attrition bias (stronger commitment agreement), confirmation bias (monitor of agricultural practices and standardized measurement), selection bias (random sampling, location and subject selection), and sample bias (disproportionate stratified sampling to ensure unbiased sample size), etc in multiple parts of the study design.

[8] # interventional study: I provide a detailed and rigorous process to control confounding variables to ensure the result is due to the difference of the independent variable between 2 groups instead of by chance or third factors. I address and justify ethical considerations. I explained the detailed process of RCT and measure the dependent variable. I identified and proposed solutions to mitigate multiple types of biases.

research could conduct partial replication to test the validity with a longer timeframe or larger sample size. They could also test the generality by experimenting with different locations/ different companion plant combinations. They could also conduct long-term studies to assess the sustainability and resilience of polyculture toward climate change and other environmental stressors.

Word count: 1298 words

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