

ABSTRACT

BATEMAN, MELANIE LYNN. Impact of Plant Suitability, Biogeography, and Ecological Factors on Associations between the Specialist Herbivore *Heliothis subflexa* G. (Lepidoptera: Noctuidae) and the Species in its Host Genus, *Physalis* L. (Solanaceae), in West-Central Mexico. (Under the direction of Fred Gould.)

Caterpillars of the moth species *Heliothis subflexa* G. (Lepidoptera: Noctuidae) are known to feed exclusively on fruits of plants in the genus *Physalis* L. (Solanaceae). However, data were lacking on whether *H. subflexa* is constrained to feeding on a subset of the approximately 90 species in this plant genus. The purpose of this research has been to determine which *Physalis* species are utilized by *H. subflexa* and to assess the relative importance of plant nutritional suitability, biogeography, and ecological factors in shaping the realized host range of *H. subflexa*. This was accomplished through a combination of field observations of plants of 17 *Physalis* species occurring at 76 field sites in West-Central Mexico, a common garden study, and laboratory bioassays. Variation among *Physalis* species in their biogeography, life history, fruit traits, and concentrations of essential fatty acids were characterized to determine if these factors impact *H. subflexa* host use.

The realized host range of this specialized herbivore proved to be constrained to thirteen of the seventeen *Physalis* species examined in this study. *Heliothis subflexa* varied with respect to the frequency and the intensity with which it infested these thirteen host species. Although *H. subflexa*'s potential host range included species that it did not infest in the field, some of the potential hosts were suboptimal for larval development, and survivorship on these *Physalis* species did not differ from survivorship on fruits of a non-*Physalis* species, *Nicandra physalodes*, which is not infested by *H. subflexa*. Positive correlations found between patterns of host use in the field, the common garden, and the laboratory bioassays indicates that plant suitability to larvae and attractiveness to ovipositing females are important determinants of *H. subflexa*'s realized host range. *Heliothis subflexa* associations with *Physalis* species are also influenced by biogeographical factors. Differences in host associations at low versus high elevations may be associated with contemporary population isolation and differentiation.

IMPACT OF PLANT SUITABILITY, BIOGEOGRAPHY, AND ECOLOGICAL FACTORS
ON ASSOCIATIONS BETWEEN THE SPECIALIST HERBIVORE *HELIOTHIS SUBFLEXA*
G. (LEPIDOPTERA: NOCTUIDAE) AND THE SPECIES IN ITS HOST GENUS, *PHYSALIS* L.
(SOLANACEAE), IN WEST-CENTRAL MEXICO

by
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BIOGRAPHY

Melanie Bateman comes from a long line of outdoor enthusiasts and gardeners. Her grandparents on both sides of her family would have made excellent botanists and field biologists. Enthusiasm for plants and the outdoors did not skip a generation. Melanie's parents took her and her brother on camping and hiking trips throughout their childhoods. She has fond memories of collecting plant material for her parents' wildflower garden. Her first job was at a plant nursery. Given her lineage and the environment in which she was raised, Melanie's subsequent career path in biology came naturally to her.

Melanie earned a B.S. in Biology from Duke University. During her undergraduate studies, she carried out independent study projects in the laboratories of Drs. Mark Rausher, Paul Manos, and Janis Antonovics with the guidance of Nora Underwood and Matt Olson. Through a summer program at the Rocky Mountain Research Laboratory in Gothic, Colorado, she had the opportunity to fly in an airplane for the first time and to get to know the American West. She also spent a semester abroad studying ecology and culture in Venezuela. After college, she worked as a research assistant for Drs. Phyllis Coley and Tom Kursar at the Smithsonian Tropical Research Station on Barro Colorado Island, Panama.

During her Ph. D. studies, Melanie moved over ten times. Each time that she packed her bags, she set off on another exciting international research opportunity. The bulk of her Ph. D. research was conducted over three years in Jalisco, Mexico. During her first two years of study in Mexico, she got a taste of big city life by living in apartments in Guadalajara. In those first two years of the project, her home base was the Centro de Ciencias Biologicas y Agropecuarias of the Universidad de Guadalajara. In her third year of study in Mexico, she was based at the Estación de Biología Chamela, UNAM, on the Pacific Coast of Jalisco. Following her last year of study in Mexico, she received a scholarship from the Toepfer Foundation which allowed her to conduct research on the fatty acid concentrations in the fruits of *Physalis* at the Max Planck Institute in Jena, Germany. Now she awaits her next adventure.

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INTRODUCTION

Why are there so many species?

As a special feature in Science magazine's 125th Anniversary Issue, the biggest gaps in our knowledge were identified, and the question "What determines species diversity?" ranked near the top of the list (Pennisi 2005). Darwin first framed this question explicitly in evolutionary terms in his book *On the Origin of Species* (Darwin 1859), and since then it has remained one of the fundamental questions driving the study of evolution. Adaptive radiation following the introduction to and exploitation of new ecological niches has been proposed as a major mechanism driving phylogenesis (Simpson 1953), and, to test this hypothesis, scientists have sought to examine key innovations that appear to have propelled diversification events.

Both the angiosperms and the insects are species rich, and phytophagous insects in particular account for approximately one quarter of all species (Janz *et al.* 2006). While fossil evidence indicates that most major plant and insect lineages predate the insects' association with angiosperms (Labandeira and Sepkoski 1993), diversification below the level of family may have been impacted by plant-herbivore interactions (Mitter *et al.* 1991). Phylogenetic studies indicate that phytophagous clades have had higher diversification rates than their non-phytophagous sister clades (Mitter *et al.* 1988, Farrell 1998). Thus, phytophagy may have been a key innovation associated with the adaptive radiation of insect herbivores and their host plants. While it has been proposed that pollinators and seed dispersers have played a major role in the adaptive radiation of the angiosperms (Dilcher 2000), the relationship between phytophagous insects and their host plants is more intimate and specialized. The majority of insect herbivores only feed on specific tissues of select plant taxa, which suggests a potential role of host specialization in the genesis of biodiversity (Dethier 1954, Ehrlich and Raven 1964, Farrell and Sequeira 2004). The exact nature of the relationship between dietary specialization by insect herbivores and the species richness of phytophagous insects and their food plants has yet to be resolved. A greater understanding of the interactions between specialist herbivores and their host plants should provide more accurate insight into the evolutionary processes that have generated biodiversity.

The fossil record includes examples of both stasis and change at different rates (*e.g.* punctuated equilibrium, gradual change without lineage splitting, and gradual lineage splitting over time; Rose and Brown 1984, Benton 1988, Jablonski and Sepkoski 1996). The presence of transitional fossils provides support for the view that macro evolutionary and micro evolutionary changes can be essentially the same process, differing only in the time scale over which they take place. Macro evolutionary patterns suggest that divergence in host use may have led to speciation and micro evolutionary studies have supported this by documenting the evolution of host races

and incipient species (Bush 1969, Feder *et al.* 1998, Funk *et al.* 2002). Thus, historical evidence, phylogenetic analyses, and ecological studies of contemporaneous interactions are all valid and potentially synergistic approaches through which the evolution of herbivore host range can be studied.

How is herbivore host range constrained?

Most phytophagous insects specialize on a closely related subset of the available plants species (Ehrlich and Raven 1964, Janz and Nylin 1998). Plant characteristics such as secondary metabolites (and, to a less understood extent, nutrient content) act as barriers to feeding by all but adapted herbivores (Dethier 1954, Scriber and Feeny 1979). A process of coevolution has been proposed in which plants have accumulated and diversified traits that confer resistance to herbivores, and specialized herbivores in turn have overcome plant defenses through a combination of physiological and behavioral adaptations (Futuyma and MacCafferty 1986). For example, the classes of compounds present in plants are phylogenetically constrained, but, within a taxonomic group, basic compounds have been elaborated into more biosynthetically derived compounds (Wink 2003). These derived changes in chemical structures can be associated with higher levels of toxicity that allow plants to escape even highly adapted herbivores (*e.g.* Berenbaum and Feeny 1981). It has been proposed that the process of reciprocal adaptation between phytophagous insects and their food plants has been an “important mechanism driving a steady increase in plant and insect diversity over the broad sweep of the fossil record” (Strong *et al.* 1984).

Other studies make the argument that herbivores have exerted little influence on the evolutionary trajectory of their host plants because selection pressures exerted by herbivores are weak and because angiosperm diversification predates the insect’s associations with plants (Jermy 1984). Consequently, herbivore’s associations with their food plants represent colonization events rather than reciprocally mediated adaptive radiations (Jermy 1984). This scenario still leaves room for host associations to have played a role in the diversification of phytophagous insects. Examination of transitions between specialization and generalization in several different taxonomic groups of insect herbivores indicated a weak directional tendency towards specialization, but transitions from specialization to generalization have also occurred, indicating that specialization is not a dead end (Nosil 2002, Nosil and Mooers 2005). Studies on the Nymphalidae and their food plant taxa suggest that “evolutionary tinkering” (transitions between generalization during host expansions and specialization) propelled the diversification of phytophagous insects (Janz *et al.* 2006). In a sister group comparison of *Polygonia* butterflies,

clades that only feed on plants belonging to the ancestral host group were less species rich than clades with expanded host ranges including plant taxa from outside of the ancestral host group (Weingartner *et al.* 2006).

While host suitability is clearly an important factor constraining herbivore's diet, other factors that influence host ranges have been detected. Restricted host ranges may be associated with an herbivore's intrinsic neurological or chemosensory limitations (Jermy 1984, Jermy 1988, Futuyma and Moreno 1988, Bernays 2001). Extrinsic abiotic and biotic factors that impact host use have also been identified. Both the historical and contemporary biogeography of the herbivore and its hosts may shape present day interactions (Thompson 1994, Feder *et al.* 2005). A comparison of the geographic ranges of butterflies and their host plants in Great Britain found that butterfly species tended to occupy only a small proportion of the geographic ranges of their food plants, they tended to be more closely associated with the most widely distributed species in their host taxa, and they occurred disproportionately in areas of high food plant species' richness (Quinn *et al.* 1998). The geographic ranges of some herbivores are larger than that of the plant species included in their host ranges, and, where multiple potential food plant species are present, the herbivore's preference may depend on the context in which the plants occur (Fox and Morrow 1981).

Selection intensity on one host species can be modulated by the presence of a chemically distinct alternate host (Zangerl and Berenbaum 2005). Across an herbivore species' geographic range, populations can be locally adapted to different host species (Hare and Kennedy 1986). Seasonal and localized environmental conditions can mediate herbivore host use (Chew 1975, Cappuccino and Kareiva 1985). Interactions with natural enemies or competitors may also promote specialization (Bernays and Graham 1988). Herbivores may avoid certain potential host plants where predation rates are higher than on other host plants (Bernays and Graham 1988, Feder 1995, Barbosa *et al.* 2001, Singer 2001, Lill *et al.* 2002), and specialists may evolve adaptations for taking advantage of refuges from natural enemies on a few specific host plants (Oppenheim and Gould 2002). The degree to which each of these factors constrains diet breadth requires further study.

Case studies as tools in the study of plant-herbivore interactions

It has been suggested that reciprocal adaptations between phytophagous insects and their hosts are the exception rather than the rule, but this suggestion may be premature and overly influenced by our inability to detect these interactions due in part to the frame of reference used in many studies. "Specialization" itself is defined on a sliding scale with highly polyphagous species at

one end of the scale, species that feed exclusively on one species at the other end of the scale, and many gradations in specialization between the two extremes (Jermy 1988). Given that much of the skew in diversity and interactions of ecological significance occur at the level of genus or species, comparative studies that focus on higher taxonomic levels may overlook important patterns. Insect herbivores and their food plants that are located at the tips of a phylogenetic tree are expected to display exceptional behavioral and physiological adaptations that are lacking in close relatives which are excluded from the interaction (Mitter *et al.* 1991), however such evidence has been found in few cases. Attempts to unravel the coevolution of interacting species have been most successful for systems with a high degree of specialization (*e.g.*, Farrell and Mitter 1990, Berenbaum 2001), or where there are morphological and behavioral traits that are required to maintain an intricate trophic interaction (Weiblen 2002, Pellmyr 2003). The development of more case studies involving systems that fit this profile should generate valuable information on the factors that constrain plant-herbivore interactions. The major goal of my thesis was to use a specific group within the Lepidoptera in developing a rigorous assessment of factors influencing host range evolution.

Fossil evidence indicates that specialized Lepidopteran associations with angiosperms date back to the initial stages of the angiosperm radiation (Labandeira *et al.* 1994), and sequence analysis of the *period* gene indicates that taxa in more derived Lepidopteran families radiated at the same time as the angiosperms during the late Cretaceous and early Tertiary (Regier *et al.* 1998). Patterns of host use across a broad sample of potential host plants have been examined in detail for groups such as the butterflies (*e.g.* Ehrlich and Raven 1964, Nylin and Janz 1998, Janz *et al.* 2001, Wahlberg 2001) but less comprehensively for other Lepidoptera families. While the Papilionoidea are conspicuous and charismatic subjects of study, other groups such as the Noctuidae are more diverse both at the level of genera (2,000 Papilionoidea versus 7,250 Noctuidae genera) and at the level of species (14,000 species versus 70,000 species respectively; Kristensen 1999). Overall, the Noctuids account for 40% of all Lepidoptera species (174,250 species and 16,298 genera). Given this high level of diversity, relationships between the Noctuids and their host plant merit further research.

Because the Solanaceae is the most important family giving rise to vegetable crops, it has been studied extensively. The family Solanaceae is made up of over three thousand species. The earliest fossil Solanaceae date to the Miocene, but phylogenetic evidence indicates that Solanaceae diversification occurred from the late Cretaceous to the early Tertiary (Knapp 2002). The fleshy fruits that are characteristic of many Solanaceae species first appeared 82 mya. This plant family has an equatorial center of origin, but Solanaceae species have radiated into a diverse

array of habitats, including rain forests, deserts, and alpine environments that experience subzero temperatures. Phylogenetic studies document the taxonomic relationships within the Solanaceae at the level of species and genera (Olmstead *et al.* 1999). Genomic studies of petunias, tomatoes, potatoes, and peppers are underway. The secondary metabolites present in many Solanaceae have been analyzed, and their impact on insect herbivores has been assessed (Griffin and Lin 2000, Wink 2003). Despite all of this study, some relatively large Solanaceae genera have been neglected. Examination of plant-herbivore interactions in these lesser-known genera will help to form a more comprehensive picture of the evolution of insects' association with the Solanaceae as a whole.

***Heliothis subflexa* & *Physalis*, a case study**

The Heliiothinae (Lepidoptera: Noctuidae) is made up of 400 species with a worldwide distribution and a South American center of diversity (Mitter *et al.* 1993). Caterpillars of this group feed on the fruits and flowers of herbaceous plants. The majority of Heliiothinae species are specialists but a handful are generalists. Molecular studies of Heliiothinae phylogeny indicate that the generalists fall into a monophyletic clade (Cho 1995, Fang 1997). *Heliothis subflexa* (Gn.) and its sister species *H. tergemina* (Felder and Rogenfer) are nested within this generalist clade. Both species are specialists: *H. tergemina* consumes solanaceaeous plants in South America (Mitter *et al.* 1993, Morales *et al.* 2003) and *H. subflexa* feeds exclusively on fruits of plants in the genus *Physalis* L. (Solanaceae) throughout the Americas (McElvare 1941, Mitter *et al.* 1993).

The *Physalis* genus includes species of economic importance: two species are grown as food crops (*P. philadelphica* and *P. peruviana*), one species is used as an ornamental (*P. alkekengi*), and several species are noxious weeds at sites where they have been introduced by humans (*e.g.* *P. viscosa* in Australia). Of the approximately 90 species of *Physalis* that have been described (estimates range from 75 to 140), only one species (*P. alkekengi*) is native to the Old World. For the genus as a whole, five species are found exclusively in the United States, six are found only in Central America, and seventy species are restricted to Mexico, making this country the center of diversity of the genus (Waterfall 1958, 1967; D'Arcy 1991, Martínez 1993, Vargas *et al.* 2003, Whitson and Manos 2005). The geographic distribution of individual *Physalis* species ranges from cosmopolitans occurring in association with a wide range of habitats to narrow endemics that are restricted to ecological islands (Vargas *et al.* 2003). The earliest identifiable *Physalis* fossils date to the Miocene (Collinson *et al.* 1993), but more recent volcanic

activity in the Trans-Mexican Volcanic Belt (<10 million years) may have resulted in even more recent speciation events.

As with most members of the Solanaceae, the *Physalis* species that have been analyzed are rich in biologically active secondary metabolites like alkaloids (Basey *et al.* 1992, Asano *et al.* 1995, Dräeger 2004). Certain steroid derivatives such as physalins and withanolides are present only in the *Physalis* and a few allied genera (Ascher *et al.* 1981, Shingu *et al.* 1992, Baumann and Meier 1993, Waiss *et al.* 1993, Tomassini *et al.* 2000, Savchenko *et al.* 2000, Su *et al.* 2002, Nagafuji *et al.* 2004, Perez-Castorena *et al.* 2004). Concentrations of secondary metabolites differ by species as well as tissue type and age (Purushothaman and Vasanth 1988, Baumann and Meier 1993). The secondary compounds in *Physalis* have been shown to impact insects (Waiss *et al.* 1993, Elliger *et al.* 1994), but their impact on *H. subflexa* has not been evaluated. The *Physalis* species' responses to *H. subflexa* feeding are not uniform. Mature or damaged fruit often abscise (Sisterson and Gould 1999), but the frequency with which this occurs is not the same for all species (Bateman *et al. in preparation*, Petzold *et al. in preparation*). Within a plant, fruit placement (main versus lateral branches) also impacts the likelihood of abscission (Mulato *et al.* 1985).

A series of studies have demonstrated that interactions between *H. subflexa* and its *Physalis* hosts are highly specialized and involve intricate trophic interactions (Laster *et al.* 1982, Sisterson and Gould 1999, Oppenheim and Gould 2002, De Moraes and Mersch 2004). For the *Physalis* genus taken as a whole, the incidence of *H. subflexa* damage to *Physalis* fruits is higher than that of any other frugivore (Bateman *et al. in prep*). *Heliothis subflexa* caterpillars frequently consume the whole fruit, destroying the seeds. Even when the fruit is only partially eaten, feeding can result in the loss of seeds in that *H. subflexa* damage induces plants of some *Physalis* species to abscise fruits (Petzold *et al. in preparation*). Thus, this herbivore represents a significant selective force. Experimental tests using fruits of *Physalis angulata* indicate that *H. subflexa* caterpillars are physiologically adapted to *Physalis* traits that kill its close relative, *H. virescens* (Laster 1982, De Moraes and Mersch 2004). Ecological studies indicate that *H. subflexa* is behaviorally adapted to *Physalis*, too (Oppenheim and Gould 2002). As in the classical scenario presented by Ehrlich and Raven (1964), these studies also suggest that by adapting to its host plants' defenses, *H. subflexa* has also been able to co-opt its host plant's characteristics and escape pressure from natural enemies (Sisterson and Gould 1999, De Moraes and Mescher 2004). *Heliothis subflexa*'s associations with *Physalis* may be part of a broader pattern of "evolutionary tinkering" within the Heliothinae in that *H. subflexa* belongs to a diverse

taxonomic group in which specialization and generalization have arisen multiple times (Mitter *et al.* 1993).

Out of the 90 different described *Physalis* species, ecological research on this system has focused on *H. subflexa*'s interactions with only a few *Physalis* species (e.g. *P. angulata* and *P. philadelphica*). Consequently, some basic information about the relationship between this herbivore and the plants in its host genus remain unknown. It is not known if *H. subflexa*'s host range is constrained to a subset of *Physalis* species. Some *Physalis* species may not be suitable hosts of *H. subflexa*. As a defining characteristic, all species in the *Physalis* genus possess an accrescent calyx (Waterfall 1957), but there is also dramatic species-to-species variation in other plant characteristics and in the species' biogeographic distributions. Intra-generic variation in plant characteristics may be associated with differential resistance to *H. subflexa*. There may be geographic variation in *H. subflexa*'s host use. *Heliothis subflexa* has been documented near the northern and southern extremes of the geographic range of its host genus, but it is not known whether their ranges overlap completely. Host preferences may depend on the *Physalis* species that are present at a given site. Ecological factors such as predators and competitors may impact *H. subflexa*'s host use. Although plants are not passive food sources, the relationship between *Physalis* species' characteristics, habitat associations, and their life histories have been almost completely ignored in studies to date. Allocation to defenses against herbivores such as *H. subflexa* may be related to trade-offs associated with investment in growth, reproduction, and seed dispersal. The balance of these trade-offs may be determined by the environment in which the plant species occurs. Thus, the purpose of this research has been two-fold: (1.) to characterize *H. subflexa*'s larval host use and to identify any physiological, biogeographic, or ecological factors that impact the relationship between the specialist herbivore *Heliothis subflexa* (Lepidoptera: Noctuidae) and plants in its host genus *Physalis* (Solanaceae), and (2.) to evaluate intra-generic variation in *Physalis* biogeography, life history strategies, and concentrations of essential fatty acids.

OBJECTIVES:

1. To compare *Heliothis subflexa*'s realized and potential host ranges through a combination of field observations, common garden studies, and laboratory assays, and to determine if differences between the realized and potential host ranges can be explained by constraints associated with plant suitability, biogeography, and ecological factors.
2. To evaluate variation within the *Physalis* genus by

- a. Quantitatively characterizing *Physalis* biogeography at the level of individual species, co-occurring species assemblages, and the genus as a whole.
- b. Comparing the life history strategy and fruit traits of 11 *Physalis* species, and assessing the relationship between these plant traits and the species' geographic distributions.
- c. Quantifying the concentration of the essential fatty acids by tissue type, age, and *Physalis* species.

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2. IMPACT OF PLANT SUITABILITY, BIOGEOGRAPHY, AND ECOLOGICAL FACTORS
ON ASSOCIATIONS BETWEEN THE SPECIALIST HERBIVORE *Heliothis subflexa* G.
(Lepidoptera: Noctuidae) AND ITS HOST PLANTS IN THE GENUS *Physalis* L. (Solanaceae)

Abstract. Caterpillars of *Heliothis subflexa* G. (Lepidoptera: Noctuidae) develop exclusively on fruits of plants in the genus *Physalis* L. (Solanaceae). The purpose of this study was to evaluate the relative influence of plant suitability, biogeography, and ecological factors on host use by this specialist herbivore. We characterized *H. subflexa*'s realized and potential host range within a set of 17 *Physalis* species through a combination of field observations at 76 field sites in West-Central Mexico, a common garden host use experiment, and no-choice bioassays of caterpillar survivorship on fruits of the different *Physalis* species.

We found that this specialized herbivore is constrained to a subset of species in its host genus. *Heliothis subflexa* infested plants of 13 of the 17 species that were examined in the field. Caterpillar performance in the laboratory assays was poor on the four species that were not part of *H. subflexa*'s realized host range in the field: no caterpillars survived on fruits of one of the *Physalis* species, and survivorship of caterpillars given fruits of the three other species did not differ from survivorship of caterpillars given fruits of the non-host species *Nicandra physalodes*. Overall, *H. subflexa*'s realized host range measured in the field was correlated to its potential host range as measured in the common garden and in no-choice bioassays, indicating the plant suitability is an important factor. The timing of mortality in the laboratory assays suggests that host suitability is determined by action of toxins or feeding deterrents rather than cumulative effects such as nutrient deficiencies.

The biogeography of *H. subflexa* and its host plants also affects this herbivore's realized host use. Within West-Central Mexico, *H. subflexa* was not excluded from any particular environment, and at least some individuals of all but two species occurred at sites where *H. subflexa* was present. Infestation to the 13 species that made up *H. subflexa*'s realized host range differed by elevation. One set of *Physalis* species that was more heavily infested by *H. subflexa* at sites below 500 m in elevation and another set of species was more heavily infested at sites above 500 m, even though plants of some species in each group occurred at sites continuously from sea level to over 1,800 m in elevation. Overall, a very strong location by *Physalis* species interaction influenced the level of infestation of a plant at a specific site more than the species itself. The species of *Physalis* that appears to be most preferred and suitable based on observations at one field site may appear much less preferred at other field sites. We discuss a number of factors that could cause this interaction.

Key words. host range, herbivory, host suitability, specialization, Mexico, biogeography, *Heliothis subflexa*, *Physalis*

INTRODUCTION

Most insect herbivores are dietary specialists both in terms of the plant taxa and the plant tissues on which they feed (Dethier 1954, Ehrlich and Raven 1964, Janz *et al.* 2001, Farrell and Sequeira 2004). Identification of the factors that limit herbivore host use has been one of the central issues in the study of plant-herbivore interactions. Plant characteristics such as chemical and physical defenses or deficiencies in essential nutrients act as barriers to all but adapted insect herbivores (Dethier 1954, Scriber and Feeny 1979, Farrell and Mitter 1994). The classes of chemical compounds that are present in plant tissues are phylogenetically constrained, but, within a taxonomic group, basic compounds have been elaborated into biosynthetically more derived structures (Wink 2003). Alterations in chemical structures can be associated with higher levels of toxicity that preclude feeding by even the most highly adapted herbivores (*e.g.* Berenbaum and Feeny 1981).

Although host suitability (as determined by plant chemistry and nutrient content) is an important constraint on herbivores' host ranges, in many cases it does not fully explain observed patterns of host use. Herbivores' realized host ranges as indicated by field observations are often smaller than their potential host ranges as measured in laboratory assays of feeding and survivorship (Bernays and Graham 1988). This finding suggests that factors in addition to plant suitability also mediate dietary specialization. Both the historical and contemporary biogeography of herbivores and their hosts may shape present day interactions (Thompson 1994, Feder *et al.* 2005). Seasonal and localized environmental conditions can influence herbivore's host use (Chew 1977, Cappuccino and Kareiva 1985). A comparison of the geographic ranges of butterflies and their host plants in Great Britain found that butterfly species tended to occupy only a small proportion of the geographic ranges of their food plants, they tended to be more closely associated with the most widely distributed species in their host taxa, and they occurred disproportionately in areas of high food plant species' richness (Quinn *et al.* 1998). The geographic ranges of some herbivores are larger than that of the plant species included in their host ranges, and, where multiple potential food plant species are present, the herbivore's preference may depend on the context in which the plants occur (Fox and Morrow 1981). The intensity of selection on an herbivore to a host species can be modulated by the presence of a chemically distinct alternate host (Zangerl and Berenbaum 2005). Across an herbivore species' geographic range, populations can be locally adapted to different host species (Hare and Kennedy 1986). Ecological interactions with natural enemies (Bernays and Graham 1988) or competitors may also promote specialization. Herbivores may avoid certain potential host plants where predation rates are higher than on other host plants (Feder 1995, Barbosa *et al.* 2001, Singer 2001,

Mira and Bernays 2002, Lill *et. al.* 2002), and specialists may evolve adaptations to exploit refuges from natural enemies on a few specific host plants (Oppenheim and Gould 2002). The degree to which each of these factors constrains diet breadth remains a matter of study.

Caterpillars of the specialist herbivore *Heliothis subflexa* Guenée (Lepidoptera: Noctuidae) feed exclusively on the fleshy berries of plants in the genus *Physalis* L. (Solanaceae) (McElvare 1941). Out of the approximately 90 (estimates range from 70 to 140) different described species of *Physalis*, ecological research on this system has focused on *H. subflexa*'s interactions with only a few species (*e.g.* *P. angulata* and *P. philadelphica*), and no study has examined host use by this herbivore in a biogeographic context. Consequently, some basic information about the relationship between this herbivore and the plants in its host genus remains unknown. Is *H. subflexa*'s constrained to feeding on a subset of *Physalis* species, and, if so, why is it restricted? As a defining characteristic, all species in the *Physalis* genus possess an accrescent calyx (Waterfall 1958), but there is dramatic species-to-species variation in *Physalis* species' characteristics, biogeography, and ecological interactions. This intra-generic variation may be associated with differences in resistance to *H. subflexa*. The aim of this study was to characterize *H. subflexa*'s larval host use and to assess the relative influence of *Physalis* species' suitability, biogeography, and ecological factors on the host range of *H. subflexa*.

To separate the respective influence of host suitability, biogeography, and ecological interactions on *H. subflexa*'s host range, we compared the realized and potential host range of this herbivore through a combination of field observations, a common garden study, and laboratory bioassays:

- (1.) We gauged natural *H. subflexa* infestation of wild and agricultural plants of 17 different *Physalis* species occurring at 76 field sites in West-Central Mexico. We assessed the geographic distribution of *H. subflexa*, and we evaluated geographic variation in its host associations. We tested whether *Physalis* species had an effect on the percentage of infested plants per site or the level of infestation to an individual.
- (2.) The *H. subflexa* insects and the *Physalis* plants observed in our field study experienced different environments and were present at varying densities. To observe host preference without the confounding factor of environmental variation, we conducted a common garden study using plants of seven *Physalis* species. As in the field study, we tested for a species' effect on the incidence and intensity of *H. subflexa* infestation.
- (3.) In both the field and the common garden, oviposition preferences of *H. subflexa* females and interactions with agents such as competitors or predators potentially influenced larval density on a given *Physalis* species. No-choice bioassays were used to directly test the

physiological limits of the caterpillars' ability to feed and survive on fruits of 14 *Physalis* species.

METHODS

Study organisms

Heliothis subflexa. – The approximately 400 species that make up the Heliiothinae (Lepidoptera: Noctuidae) have a worldwide distribution with a South American center of diversity (Mitter *et al.* 1993). Caterpillars of this group feed on the fruits and flowers of herbaceous plants. The majority of Heliiothinae species are specialists but a handful are generalists. Molecular studies of Heliiothinae phylogeny indicate that the generalists fall into a monophyletic clade (Cho *et al.* 1995, Fang *et al.* 1997). The specialist species *H. subflexa* is nested within this generalist clade. The geographic range of *H. subflexa* extends from Wisconsin (G. Bush, pers. obs.) to Peru (J. Cabrera, pers. obs.), but it is not known whether it is excluded from any geographic areas between these two points.

Larvae of *H. subflexa* feed exclusively on fruits of plants in the genus *Physalis* (McElvare 1941, Sheck and Gould 1993). For many *Physalis* species, the incidence of *H. subflexa* damage to *Physalis* fruits is higher than that of any other frugivore (Bateman *et al.* in prep). *Heliothis subflexa* caterpillars frequently consume the whole fruit, destroying all of the seeds. Some *Physalis* species abscise damaged fruits even when the fruit is only partially eaten (Petzold *et al.* in preparation). The abscission of immature fruits results in the loss of all of that fruit's seeds. Thus, *H. subflexa* can be an important selective force on its host plants. Although moths of *H. subflexa* occasionally feed on the nectar of *Physalis* flowers (N. D. Benda, unpublished data), the adults do not have an obligate relationship with *Physalis* in terms of feeding or mating. Females of *H. subflexa* lay their eggs singly on the reproductive parts, leaves, and stems of *Physalis* plants and occasionally on neighboring non-host plant species (N. D. Benda, unpublished data). To feed, each *H. subflexa* caterpillar cuts a small hole in the inflated calyx and then bores into the fruit. Although other Lepidopteran, Dipteran, and mammalian agents occasionally feed on *Physalis* fruits (Bateman *et al.* in preparation), stereotypical damage by *H. subflexa* differs from that of these other frugivores (*e.g.* *H. subflexa* caterpillars bore into the fruit but larvae of *Lineodes fontanella* [Lepidoptera: Crambidae] feed by scraping the inside of the calyx and the fruit surface; Bateman in preparation). While on the outside of the calyx, *H. subflexa* caterpillars are exposed to natural enemies, but, once inside the calyx, they are sheltered from natural enemies such as predators and parasitoids (Sisterson and Gould 1999). Typically, *H. subflexa* caterpillars feed on more than one fruit before completing larval development, and the

timing of their movements does not depend on the time of day or the amount of fruit consumed (M. E. Puente, unpublished data). At the end of larval development, *H. subflexa* larvae move off of the plant and pupate in the soil.

Physalis genus. – Along with other comparatively well-studied genera like *Capsicum*, *Datura*, and *Solanum*, the *Physalis* genus is placed in the subfamily Solanoideae (Olmstead *et al.* 1999). An enlarged calyx is typical of many Solanoideae (*e.g.* *Nicandra*, *Jaltomata*) (Knapp 2002), but the degree to which the calyx encloses the fruits of all species in the genus *Physalis* is extreme (Martínez 1998, Whitson and Manos 2005). With its fleshy but cryptic berries, *Physalis* species fit the profile of plants with mammalian seed dispersal (Van der Pijl 1969). While the genus as a whole is paraphyletic, the morphologically typical *Physalis* species form a monophyletic clade (Whitson and Manos 2005). This study of *H. subflexa*'s host use focuses on 13 annual and 4 perennial species in the morphologically typical *Physalis* clade (Table 1).

While the *Physalis* genus is united by the accrescent calyx, there is dramatic species-to-species variation in other traits (Estrada and Martínez 1999). The general plant architecture and the surface pubescence of the species vary (Seithe and Sullivan 1990, Martínez 1993, Vargas *et al.* 2003). The species also differ with respect to life history strategies (refer Chapter 5) and the form, size, and color of their fruits and calyces (Menzel 1951, Martínez 1993, Vargas *et al.* 2003). The concentrations of essential nutrients differ both by species and by fruit maturity (Ramadan and Mörsel 2003, Deineka and Deineka 2004, De Moraes and Mescher 2004; see also Chapter 4).

As with most members of the Solanaceae, the *Physalis* species that have been analyzed are rich in biologically active secondary metabolites such as alkaloids (Basey *et al.* 1992, Asano *et al.* 1995, Dräeger 2004). Certain steroid derivatives such as physalins and withanolides are present only in the *Physalis* and a few allied genera (Ascher *et al.* 1981, Shingu *et al.* 1992, Baumann and Meier 1993, Waiss *et al.* 1993, Tomassini *et al.* 2000, Savchenko *et al.* 2000, Su *et al.* 2002, Nagafuji *et al.* 2004, Pérez *et al.* 2004). Concentrations of secondary metabolites differ by species as well as tissue type and age (Purushothaman and Vasanth 1988, Baumann and Meier 1993). The secondary compounds present in *Physalis* fruits have been shown to have a negative effect on some insect species (Waiss *et al.* 1993, Elliger *et al.* 1994), but their impact on *H. subflexa* has not been evaluated. Damaged fruit often abscise in response to larval feeding (Sisterson and Gould 1999), but the frequency with which this occurs is not the same for all species (Petzold *et al. in preparation*). Within a plant, fruit placement (main versus lateral branches) also impacts the likelihood of abscission (Mulato *et al.* 1985).

The *Physalis* genus occurs primarily in the New World. Seventy of the approximately 90 described *Physalis* species are restricted to Mexico, making this country the center of diversity of the genus (Waterfall 1958, 1967; D'Arcy 1991, Martínez 1993). Whereas in many regions of the world *Physalis* species tend to be rare and patchily dispersed, in Mexico the plants are relatively common in all but the most extreme environments (Martinez 1993, Vargas *et al.* 2003; see also Chapter 3). Thirty-nine species of *Physalis* occur in West-Central Mexico. The biogeography of the 17 *Physalis* species examined in this study varies (Vargas *et al.* 2003). The number of sites at which a species occurred in West-Central Mexico was positively correlated with the size of the species' overall geographic range and the breadth of the habitat gradient across which the species occur (see Chapter 3). Seven of the nine most common and widespread species frequently co-occur. Five of these species are cosmopolitan, ranging throughout the Americas and secondarily introduced to the Old World. A second group of species tend to occur in lowland coastal locations, and a third is restricted to the Central Mexican Matorral. Two species are narrow endemics that only occur in the Trans-Mexican Volcanic Belt (Martínez 1993, Vargas *et al.* 2003).

The fruit of many *Physalis* species are edible, and some species have been grown agriculturally for thousands of years (Jenkins 1948, Martínez 1993, Montes and Aguirre 1994). In present-day Mexico, the tomatillo (*Physalis philadelphica*) is used in salsas, and it is grown commercially in almost every state in the country (SAGARPA, 2002). The tomatillo comes in two distinct forms: large-fruited “tomate de cascara” or “husk tomato” which is produced by commercial seeds and small-fruited landrace plants called “miltomate” (Hudson 1986, Martínez 1993, Montes and Aguirre 1994). Typically, there are high levels of gene flow between milpa plants and their wild relatives (Lira 1995, Brush and Tadesse 2003).

Field assessment of H. subflexa realized host range

Study area and field sites. – Important natural features in West-Central Mexico that affect the environment of the site examined in the study include the narrow band of the Pacific Coastal lowlands, the Occidental Sierra Madre, the Trans-Mexican Volcanic Belt, and the Central Mexican Plateau (Rzedowski 1978, Olson and Dinerstein 1998, Olson *et al.* 2001). The climate of sites at the same elevation can differ substantially (Bobbink and Heil 2003).

In 2002 and 2003, we examined *H. subflexa* infestation of fruiting *Physalis* plants at 76 field sites between 18° – 23° North and 101° – 105° West in the West-Central Mexican states of Aguascalientes, Colima, Jalisco, Michoacán, and Zacatecas, (Fig. 1, Appendix A at end of thesis; for a detailed examination of *Physalis* biogeography at the field sites, refer to Chapter 3). Using

GPS units (Garmin Etrex Summit), we acquired the geographic coordinates and altitude of each location. We examined accessible locations where *Physalis* was likely to be present. These sites were located along most of the major roads, many of the secondary roads, and some unimproved dirt roads mostly in Jalisco and Colima but also in portions of the neighboring states. These sites had moderate levels of disturbance, typical of the habitats in which *Physalis* occurs (e.g. roadsides, trails, pasture, stream banks, coastal dunes, cornfields, forest edges, and tomatillo fields; Waterfall 1958, Vargas *et al.* 2003). The field sites were located in each of the major ecoregions (see Chapter 3). The elevation of the study sites ranged from sea level to 2,400 m. We examined cultivated plants at 14 tomatillo farms. Wild *Physalis* plants were found at 62 sites with completely natural plant populations and at nine of the tomatillo farms. Over the three year study period, we took data on one occasion only at 43 field sites. However, at 40 of the field sites we collected data on multiple occasions (two to eight times) over the course of the wet season and the early part of the dry season (refer to Appendix A for more detailed descriptions of the field localities). The set of sites that we revisited included locations for which we did and did not encounter infestation by *H. subflexa* during the first round of data collection.

Period of field study. – In West-Central Mexico, seasonal change in the abundance of herbaceous plants tends to be associated with variation in rainfall rather than temperature. The rainy season runs from May to October. Most wild *Physalis* plants begin fruiting in the early rainy season and senesce following the onset of the dry season (Vargas *et al.* 2003), but on irrigated farms tomatillos are also grown in the dry season. Over the course of the three years, we took data in the field on a total of 81 days spanning from August 6th through November 29th (Table 2). As of the latter half of October, wild plant populations were in decline. We continued to take data on plants growing on tomatillo farms until late November.

Data collection in the field. – Because *Physalis* plants in wild populations differed dramatically in distribution and density from those on tomatillo farms, two different protocols were followed in surveying plants from these two types of populations. At the sites with wild *Physalis*, we walked in a random direction, and we took data approximately every 5 m on a randomly selected *Physalis* plant, without reference to plant size. We searched for plants across a minimum area of 200 m² for at least 15 minutes. Whenever possible, we examined 24 wild plants per data collector per field site per date, with each species evenly represented. In tomatillo monoculture, we started at a random point in the field, walked 20±2.5 meters along a row, selected a random

plant, and then shifted rows. Within this framework, we aimed at collecting data on tomatillo plants throughout the field.

The method of data collection for each selected plant was the same in both types of plant populations. We noted whether or not each plant was in fruit and infested by *H. subflexa*. To detect *H. subflexa* infestation, we scanned the calyces for *H. subflexa* entry holes. We then opened the calyx and examined the fruit for *H. subflexa* larvae and/or damage that was characteristic of *H. subflexa*. We counted all of the attached and abscised fruits with and without *H. subflexa* damage that were associated with each plant. We also counted the number of *H. subflexa* larvae feeding on the fruits of each plant.

Common garden assessment of H. subflexa's potential host range

Common garden location.— The common garden experiment was conducted at the Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA) of the Universidad de Guadalajara, Zapopán, Jalisco, Mexico (1,550 m in elevation). This location has a semi-arid tropical climate (Tereshchenko and Filonov 2001). Crops under cultivation in neighboring field plots included tomatillos, squash, and corn. Natural populations of *Physalis* and *H. subflexa* also occur at this location.

Common garden plants. — A common garden study was conducted to compare *H. subflexa* infestation on plants of seven of the nine most common annual species of *Physalis* in West-Central Mexico (*P. ampla*, *P. angulata*, *P. lagascae*, *P. minuta*, *P. nicandroides*, *P. philadelphica*, and *P. pubescens*; Table 1). Six of the species (*P. ampla*, *P. angulata*, *P. lagascae*, *P. nicandroides*, *P. philadelphica*, and *P. pubescens*) are widespread habitat generalists occurring from sea level to 2,600 m (Vargas *et al.* 2003). Plants of these six species frequently co-occur (see Chapter 3). Plants of *P. minuta* are common on beaches, sand dunes, and rocky outcrops in lowland coastal locations (Vargas *et al.* 2003), but *P. minuta* has not been observed to co-occur with the plants of the other six species (Bateman *et al. in prep.*).

Germplasm for the plants used in the common garden came from seeds and seedlings of wild and domesticated plants collected from the field in West-Central Mexico (Appendix A). Seedlings were initially kept in 10 cm deep soil-filled trays. Once they had four or more true leaves, the plants were transferred to 3.75 L pots. Potted plants were kept outside where they experienced natural sunlight and rainfall. They were watered as needed and remained in pots until they were approximately 50 days old, the age at which individuals of most species become reproductive (Cartujano *et al.* 1985; see also Chapter 5).

Common garden design. – On September 1st – 3rd of 2003, 72 individuals of each of the seven species were transplanted into a recently tilled garden plot. Plants were spaced 0.80 meters from each other in eight randomized blocks with nine individuals of each species per block. Two thirds of the plants of each species in each block experienced herbivore exclusion treatments, but these plants are not dealt with here. Only *H. subflexa* infestation to the 168 untreated control plants is addressed in the current study. Plants that died within the first ten days of the study were replaced. For the first two weeks following transplantation, weeds were removed from an area with a 50 cm radius around each of the experimental plants. Once a week all of the common garden plants were fertilized (1 tablespoon of NPK 15-30-15 fertilizer per gallon of water). Other than that, plants were not irrigated. The plants did not experience drought stress until the end of the wet season.

Data collection in the common garden. – Beginning September 19th and continuing on a weekly basis until each plant either died or the study ended, we took data on *H. subflexa* infestation to the common garden plants. We scanned the calyces of the fruit that were attached to each study plant for *H. subflexa* entry holes. When we encountered signs of *H. subflexa* feeding, we ascertained whether a larva was present by gently feeling the surface of the fruit through the calyx and by peering through holes in the calyx. In this way, each week we estimated how many *H. subflexa* larvae were present in the fruit that were attached to the plants. On a weekly basis, we collected all of the abscised fruit that were associated with each plant. We opened the calyces of abscised fruit, and we counted the number of abscised fruits with and without *H. subflexa* larvae and/or damage. We also counted the number of *H. subflexa* larvae feeding on the abscised fruits. When plants were showing advanced symptoms of senescence, we conducted a final census of *H. subflexa* infestation by opening the calyces and examining all of the attached and abscised fruits that were associated with each plant. When the study ended on November 14th of 2003, the rains had stopped and most of the common garden plants had either died (61.7%) or were showing symptoms of senescence. At this time, the wild *Physalis* plants that were growing in the borders of the field had senesced.

Bioassays of potential host range

Plants and insects used in bioassays. – No-choice bioassays of feeding were used to assess *H. subflexa*'s physiological ability to survive on fruits of 13 *Physalis* species: *P. ampla*, *P. angulata*, *P. cordata*, *P. lagascae*, *P. lignescens*, *P. leptophylla*, *P. minuta*, *P. nicandroides*, *P. patula*, *P. philadelphica*, *P. pruinosa*, *P. pubescens*, and *P. solanaceous* (Table 1). Each of the annual

species for which infestation was observed in the field and in the common garden was used in the bioassays. *Physalis liguenscens* was the only perennial species used in the bioassays. Fruit production of plants of the other three perennial species was too low for use in bioassays. As a positive control, *H. subflexa* performance was also tested on three artificial diets: modified corn-soy blend and Southland's Products (Burton 1970, Joyner and Gould 1985; Table 3). These artificial diets are formulated to contain optimal levels of nutrients for maximal growth rates and survivorship of Lepidopteran larvae. As negative controls, *H. subflexa* survivorship was evaluated on fruits of three non-host species from other Solanaceous genera: *Jaltomata procumbens*, *Nicandra physalodes*, and *Solanum americanum*.

Germplasm for the plants used in the bioassays came from seeds and root stock of wild and agricultural plants collected from the field in Mexico (Appendix A). The study was conducted at two locations: CUCBA and the Estación de Biología Chamela of the Universidad Nacional Autónoma de México, Jalisco, México (Table 3). At each location, the seeds were sown into 10 cm deep flats, and when the seedlings had four or more true leaves, the plants were transferred to 3.75 L pots. The potted plants were kept outside where they received natural sunlight and rainfall. The plants were watered as needed. One teaspoon of timed-release fertilizer (NPK 14:14:14) was mixed into the potting soil of each plant, and the plants were also given liquid fertilizer on a weekly basis (1 tablespoon of NPK 15-30-15 fertilizer per 3.75 L of water).

Heliothis subflexa caterpillars used in the bioassays were of Mexican origin (Table 3). In all years and at all locations, the protocol used to maintain the *H. subflexa* colonies followed that of Sheck and Gould (1993). At both Mexican research facilities, the insects were kept in open-air laboratories, and the average ambient temperature varied by 7° C (CUCBA average=20.03° C versus Chamela average=27.09° C; Table 3). Natural *H. subflexa* populations were present in the wild in the immediate surroundings of both of the Mexican research facilities, indicating that the environmental conditions at each location was within the range that *H. subflexa* will tolerate.

Bioassay protocol. – Neonates are often more susceptible to plant secondary compounds than older instars, and their ability to survive on a host plant is a critical measure of the suitability of a plant (Zalucki *et al.* 2002). Consequently, *Heliothis subflexa*'s potential host range was evaluated by placing newly emerged neonate caterpillars individually in medicine cups with fruits of one of the test species or artificial diet. Eleven of the *Physalis* species used in the bioassays tend to abscise damaged fruit, and in nature *H. subflexa* caterpillars are frequently found feeding on abscised fruit. Thus, the use of detached fruit is consistent with the conditions that caterpillars

experience in the field. The fruits used in the assays were randomly selected from multiple individuals of each plant species. The smallest fruits (less than 3 mm in diameter) were not used in the assays because they tended to desiccate rapidly. Otherwise, fruits were not selected with reference to age.

Over the course of the bioassays, the fate of each caterpillar was followed on a daily basis, and fruits were replenished as needed based on the daily inspections. We noted whether or not each caterpillar established on the test diet that they were given. Establishment was defined as feeding and surviving on the administered test diet for at least the first 96 hours following the start of the assay. Caterpillars that failed to establish typically died without feeding. For those caterpillars that established on the test diet that they were given, we noted whether or not they also persisted to pupation. Persistence was defined as feeding and surviving to pupation on the administered test diet. We also evaluated overall survivorship of the caterpillars reared on each species (the percentage of neonates given a diet that survived to pupation). For all years pooled, a minimum of 70 caterpillars were assayed per species (with the exception of *P. leptophylla* [n=48]). All 13 *Physalis* species and each of the control diets were evaluated in bioassays in 2003, but only seven *Physalis* species were assessed in bioassays in 2004.

Statistical analyses

All statistical analyses were conducted using SAS software (SAS 8.2 for Windows, SAS Institute, Cary, NC, 2001). Data are referred to as significant for $P < 0.05$ and are presented as the mean ± 1 standard error (SE). Corrections were made to significance values to account for multiple tests on a specific data set.

Field observations. – For a *Physalis* species to be a part of *H. subflexa*'s realized host range, it has to occur at sites where *H. subflexa* is present. *Heliothis subflexa* was considered to be present at a location if we detected caterpillars or damaged fruits in association with the sampled plants. Using logistic regression models, we tested whether the probability that *H. subflexa* occurred at a site was related to either the elevation or the species richness of the site. We tested whether the 17 *Physalis* species differed in the frequency with which they occurred at sites where *H. subflexa* was present using a chi-square contingency table.

For the field sites where *H. subflexa* was present, we determined which *Physalis* species were part of *H. subflexa*'s realized host range. A species was considered to be within the *H. subflexa* realized host range if upon examination of the sampled plants at all of the sites we encountered at least one *H. subflexa* caterpillar or at least one fruit with *H. subflexa* damage. For

the *Physalis* species that were part of *H. subflexa*'s realized host range, the SAS procedure GENMOD was used to carry out a logistic regression to evaluate whether *H. subflexa* infestation differed by *Physalis* species, location, date, or the *Physalis* species x date interaction (Appendix C). We accounted for the overdispersion of the data by using the *p scale* option in the model statement. Only species and locations for which we had multiple observations over multiple dates were included in the analysis.

To assess geographic variation in host use by *H. subflexa*, a Euclidean distance matrix was constructed based on whether or not plants of each *Physalis* species at each site were infested and a dendrogram was constructed using Ward's method (Sneath and Snokal 1973). To test the robustness of the identified clusters, another dendrogram was constructed using Jaccard similarity indices of infestation to each species at each site and Complete Link (Defays 1977). The distance matrices and the dendrograms were generated using ADE-4 Software (Thioulouse *et al.* 2001). For the sets of species in *H. subflexa*'s realized host range that frequently co-occur at the same locations in the field (identified using hierarchical clustering, see Chapter 2), the percentages of plants per site with *H. subflexa* infestation was compared using paired *t*-tests with sequential Bonferroni correction.

We used the Kruskal-Wallis H test to evaluate whether the number of caterpillars or the percentage of fruit with damage per infested plant differed by *Physalis* species. Whenever the overall models were significant at 0.05, we compared the plant species' means using Mann-Whitney U test with Bonferroni correction. We evaluated whether average number of caterpillars per infested plant, the percentage damaged fruit per infested plant, or the percentage of plants with infestation per site were correlated (we typically found fewer larvae than damaged fruit).

Common garden experiment. – The percentage of plants with infestation was analyzed using ANOVA with *Physalis* species as a fixed effect and block as a random error term. We used the Kruskal-Wallis H to test whether the mean number of caterpillars or the percentage of damaged fruit per infested common garden plant differed by *Physalis* species. Spearman's Rho was used to test for correlation between the percentage of common garden plants of a species with damage, the number of caterpillars per damaged plant of a species, and the percentage of damaged fruit per infested plant of a species.

Bioassays. – The laboratory colony, the laboratory conditions, and the set of test diets that were evaluated differed in each year. Consequently, analyses of caterpillar performance were carried

out separately for each year. For the set of test diets that were assayed in multiple years, cross year agreement was assessed with Spearman's Rho.

Through the bioassays, we sought to identify differences in *H. subflexa* larval performance on fruits of species in its host genus. Consequently, only *Physalis* species were included in the tests of overall significance. Chi-square contingency tables were used to test whether there was a relationship between the *Physalis* species on which the caterpillars were assayed and the caterpillars' overall survivorship, establishment, and persistence. In comparisons of persistence on a diet, only those *Physalis* species on which more than 20 caterpillars established were included in the analyses. When the overall test for difference among all *Physalis* species was significant at 0.05, pair wise comparisons were carried out among the *Physalis* test diets as well as the positive and negative controls using either chi-square or Fisher's exact test (when sample sizes in 50% of the cells were less than five) with adjustment for multiple comparisons using the Bonferroni procedure. Thus, caterpillar performance on fruits of the *Physalis* species was benchmarked to that of caterpillar performance on optimal hosts (artificial diet) and non-hosts (fruits of plants in the other genera). Whenever one of the measures of *H. subflexa*'s potential host range was zero, that test diet was excluded from the analyses because zero values made the data heteroscedastic.

Relationship between realized and potential host ranges. – We examined more species in the field and in the bioassays than in the common garden. Consequently, we used non-parametric tests to examine the relationship between *H. subflexa* infestation in comparisons of the common garden data to observations from the field or the bioassays, and we used parametric tests to examine the relationship between *H. subflexa* host use in the field and in the bioassays. Using Spearman's Rho, we tested whether the percentage of infested plants per species, the number of caterpillars per infested plant, or the percentage of damaged fruit per infested plant in the field and in the common garden were correlated. We calculated Pearson correlation coefficients for the measures of *H. subflexa* host use in the field and *H. subflexa* performance in the laboratory assays (overall survivorship, percent establishment, and persistence), and we evaluated the significance using the CORR procedure (SAS, SAS Institute, Cary, NC 2003). Since the conditions under which the bioassays took place differed by year and 2003 was the only year in which all diets were tested, the data for 2003 were used in these comparisons. The Bonferroni procedure was used to correct for multiple comparisons.

RESULTS

Field observations of Heliothis subflexa and Physalis. – The 17 *Physalis* species differed in the frequency of occurrence at the 76 field sites. Tomatillo plants were present at more locations than any other species (14 commercial tomatillo farms, eight sites with feral large-fruited plants, and ten sites with small-fruited “miltomate” plants). Seven of the wild annual species were relatively common, occurring at ten or more of the field sites, and the other eight wild species (five perennial and three annual) were relatively rare, occurring at only one to four locations. While the *Physalis* species differed with respect to the number of locations at which they were present, there was no significant relationship between the number of sites at which a species occurred and the average number of plants examined per site for each species ($r_s = -0.34$, $P = 0.18$), which confirms that data collection within a site was not biased by the overall frequency with which the *Physalis* species occurred at our set of field sites.

Heliothis subflexa was present at 51 of the 76 field sites with fruiting *Physalis* plants, and at least at some locations *H. subflexa* was present from early August to the end of November. There was no relationship between the elevation of a site and the presence of *H. subflexa* (Logistic regression: $df=1$, Chi-square=0.46, $P = 0.50$). The probability that *H. subflexa* occurred at sites was positively correlated with the *Physalis* species richness of the sites (Logistic regression: $df=1$, Chi-square=4.12, $P = 0.04$). *Heliothis subflexa* was never found at sites where *P. lignescens* and *P. patula* occurred. Plants of these two species only occurred at one site each, and no plants of other *Physalis* species co-occurred with either species. *Physalis cinerascens*, *P. glutinosa*, *P. hastatula*, and *P. orizabe* only occurred at sites with *H. subflexa* infestation, and data were collected on fruiting plants of these species at only one location each, and they were therefore not included in the statistical analysis. There were no statistical differences in the probability that plants of the other 11 species co-occurred with *H. subflexa* ($df=16$, $N=174$, Chi-square=7.15, $P = 0.71$; Fig. 2a).

Infested plants belonged to 13 different *Physalis* species. These species formed *H. subflexa*'s realized host range in the field. At least one species out of a set of nine species (*P. ampla*, *P. angulata*, *P. cordata*, *P. lagascae*, *P. minuta*, *P. nicandroides*, *P. philadelphica*, *P. pruinosa*, and *P. pubescens*) occurred at each of the 51 locations with *H. subflexa* infestation. Plants of these species also occurred at some locations where *H. subflexa* was not present. Although some plants of *P. leptophylla* ($N=4$) and *P. solanaceous* ($N=45$) occurred at sites where *H. subflexa* was present and plants of other *Physalis* species were infested, none of the sampled field plants of these two species were ever infested by *H. subflexa*.

While the majority (85.54%) of the plants in *H. subflexa*'s realized host range occurred at sites where *H. subflexa* was present, only one quarter (531 of the 2,023 plants) of the fruiting *Physalis* plants at those sites were or had been infested by *H. subflexa*. There was a relationship between *H. subflexa* infestation and *Physalis* species, location, date, and the interaction between *Physalis* species and date (Table 4; Fig. 2b). The location effect was strong, indicating that the context in which the plants occurred affected how susceptible the *Physalis* species were to *H. subflexa*. Infestation tended to increase over the course of the season for some but not all species. *Heliothis subflexa* infestation to plants at a given site ranged from 6.72%±1.63% of *P. minuta* plants to 38.6%±2.62% of tomatillo plants. The frequency with which tomatillo plants were infested differed by the context in which they occurred (df=2, Chi-square=11.92, $P = 0.003$). A significantly higher percentage of the feral large-fruited plants (57.4%) were infested than the miltomate plants (41.9%) or the large-fruited plants grown in agriculture (36.6%). These differences may be due to the use of pesticide sprays on commercial tomatillo farms.

Cluster analysis with Ward's method using Euclidean distances identified four main groups of *Physalis* species based on the similarity of *H. subflexa* infestation across the 76 field sites in West-Central Mexico (Fig. 3). The nine species that were always present at sites with *H. subflexa* infestation separated into two clearly identifiable sub-groups: a cluster of six species that tended to be infested at sites above 500 m in elevation (A) and a cluster of two species that tended to be infested at sites below 500 m in elevation (B). This reflects the species by location interaction in the ANOVA. Three species in cluster A (*P. ampla*, *P. angulata*, and *P. nicandroides*) only occurred at sites over 500 m in elevation. One of the species in cluster B (*P. minuta*) only occurred at sites below 50 m in elevation. Three other species occurred at sites ranging from sea level to over 1,800 m in elevation, but *H. subflexa* infestation of these species sorted by elevation: all infested plants of the *P. pruinosa* occurred at sites above 500 m in elevation, and all infested plants of *P. pubescens* occurred at sites above 1,550 m in elevation. *Physalis lagascae* plants were infested at only one site below 500 m, but they were infested at six sites above 500 m. These three species were in cluster A. Meanwhile, *P. cordata* (cluster B) was infested at only one out of nine sites above 500 m in elevation, and it was infested at seven out of the ten sites at which it occurs below 500 m in elevation. A third cluster (C) was composed of four species that were only infested when they co-occurred with plants in cluster A. Three of these species only grow in the Central Mexican Matorral, and the fourth species is endemic to mountain peaks in the Trans-Mexican Volcanic Belt. The non-host species formed a fourth cluster (D). Depending on the clustering method used, tomatillo plants either fell into cluster A or formed a basal branch of the nine most common hosts. Since the distribution of tomatillos is

highly influenced by human activities, it is a special case and probably does not belong to either cluster. The dendrogram produced by Complete Link using Jaccard similarity indices was virtually identical to the dendrogram generated by Euclidean distances and the Ward method.

To assess *H. subflexa*'s relative preferences for co-occurring plants of species in clusters A and B, we carried out paired *t*-tests (Table 5). On tomatillo farms, a significantly higher percentage of the tomatillo plants were infested than plants of *P. nicandroides* ($t=25.80$, $P = 0.0015$) and *P. pubescens* ($t=4.49$, $P = 0.006$). In pair-wise comparisons among *P. ampla*, *P. angulata*, and *P. pubescens* occurring on tomatillo farms, the percentage of plants with *H. subflexa* infestation was not significantly different. In wild populations, the percent of *P. lagascae* plants with infestation was significantly higher than that of plants of *P. nicandroides* plants ($t=2.77$, $P = 0.05$) and marginally higher than that of *P. pruinosa* plants ($t=4.00$, $P = 0.10$ following Bonferroni correction). In pair-wise comparisons among the other species in cluster A, the percentage of plants with *H. subflexa* infestation was not significantly different. There were no significant differences in the percentage of plants with infestation between *P. cordata* plants that co-occurred with plants of *P. minuta* or *P. philadelphica*.

A total of 492 *H. subflexa* caterpillars were found feeding on fruits of 211 plants belonging to seven different *Physalis* species. The number of caterpillars found feeding on fruits of a single plant ranged from 0 to 20. There was a significant effect of *Physalis* species on the mean number of caterpillars per plant (Kruskal-Wallis H test: $df=7$, $N=504$, Chi-square=53.08, $P < 0.0001$; Fig. 2c). There were significantly more caterpillars per plant of *P. angulata* (1.08 ± 0.36 caterpillars), *P. philadelphica* (1.19 ± 0.13 caterpillars), and *P. pubescens* (0.79 ± 0.32) than for any other species. The number of caterpillars per infested plant was not correlated with the percentage of plants per site with infestation ($P = 0.24$).

Almost one quarter (24.6% of 17,853) of the fruits associated with infested plants had been damaged. The percentage of fruit per infested plant with *H. subflexa* damage differed significantly by *Physalis* species (Kruskal-Wallis H test with only species for which ten or more plants experienced *H. subflexa* damage included in the analysis: $df=7$, Chi-square=27.29, $P = 0.0003$; Fig. 2d). The percentage of damaged fruits per infested plant was highest for *P. philadelphica* plants ($32.0\% \pm 1.45\%$) and lowest for *P. minuta* plants ($11.1\% \pm 1.86\%$). The percentage of damaged fruits per infested plant was positively correlated with the percentage of infested plants at sites where *H. subflexa* was present ($r = 0.84$, $P = 0.001$) and to the mean number of caterpillars per infested plant ($r = 0.78$, $P = 0.02$).

H. subflexa infestation in the common garden. – At least some plants of each of the seven common garden species were infested by *H. subflexa*, but almost half (44.0%) of the 125 plants that fruited were never infested by *H. subflexa* at any point during the study. The *Physalis* species and the *Physalis* species x block interaction significantly affected the percentage of common garden plants with *H. subflexa* infestation (Fig. 4a; Table 6). The percentage of plants with infestation ranged from 22.22%±14.05% for plants of *P. angulata* to 93.75%±6.25% for plants of *P. pubescens*.

Forty-seven *H. subflexa* caterpillars were found feeding on the fruits of the study plants. The total number of caterpillars found feeding on each plant ranged from 0 to 7, and no caterpillars were ever found feeding on fruits of *P. minuta* or *P. ampla*. There was no effect of *Physalis* species on the mean number of caterpillars per infested plant ($P > 0.30$; Fig. 4b).

The percentage of damaged fruit per infested plant differed by *Physalis* species (Median one-way analysis: $P = 0.03$; Fig. 4c). The percentage of damaged fruit per infested plant ranged from 8.76%±4.03% for *P. ampla* plants to 33.40%±9.03% for *P. minuta* plants. The percentage of infested common garden plants and the percentage of damaged fruit per infested plant was not correlated ($r_s = 0.60$, $P = 0.20$).

Bioassays of H. subflexa's potential host range. – There were significant annual differences in the performance of the caterpillars. Survivorship from neonate to pupation of caterpillars reared on artificial diet differed by year ($df=2$, 292; $F=31.03$; $P < 0.0001$). Survivorship was higher in 2003 (73.8%) than in 2004 (31.5%). The insects' ages at the time of pupation also differed significantly by year ($df=2$, 270; $F=77.59$; $P < 0.0001$). It took more days for caterpillars to enter the pupal stage in 2003 (35.9±1.4 days old) than in 2004 (21.0±0.7 days old). While the caterpillars took longer to reach pupation in 2003, the pupae were heavier in 2003 (0.30±0.01 g) than in 2004 (0.24±0.01 g). This amount of variation is high but often found even within one laboratory colony over time.

None of the caterpillars that were given fruits of *J. procumbens* or *S. americanum* survived to pupation, and 4.17% of the caterpillars given fruits of *N. physalodes* survived to pupation. Overall survivorship from neonate to pupation of insects given fruits of *N. physalodes* did not differ from that of caterpillars assayed on fruits of seven of the *Physalis* species (Table 7).

Overall survivorship from neonate to pupation differed by the *Physalis* species on which the caterpillars were assayed (Table 7; 2003: $df=12$, $N=899$, $Chi-square=104.52$, $P < 0.0001$; 2004: $df=2$, $N=117$, $Chi-square=6.08$, $P = 0.05$ & survivorship on four hosts was 0%). None of the caterpillars given fruits of *P. patula* survived to pupation. With one exception (*P. minuta* in

2004), the percent survivorship was higher on fruits of *P. angulata* than on fruits of any other *Physalis* species. The estimates of survivorship were not correlated in 2003 and 2004 ($r_s = 0.57$, $P = 0.14$).

At least some caterpillars established on fruits of each of the *Physalis* species that was evaluated. The percent establishment differed by *Physalis* species (Table 7; 2003: $df=12$, $N=987$; Chi-square=129.13, $P < 0.0001$; 2004: $df=6$, $N=267$; Chi-square=28.30, $P < 0.0001$). Establishment was consistently higher on fruits of *P. philadelphica* ($74.3\% \pm 1.46\%$) and lower on fruits of *P. lagascae* ($19.0\% \pm 2.33\%$). The estimates of establishment were correlated in 2003 and 2004 ($r_s = 0.79$, $P = 0.02$). Establishment of caterpillars given the artificial diet positive control ($93.1\% \pm 4.72\%$) was higher than that of caterpillars given fruits of any *Physalis* species. All caterpillars given fruits of *S. americanum* and *J. procumbens* died without feeding. Percent establishment of *H. subflexa* caterpillars given fruits of *N. physalodes* was either higher (six species) or not significantly different (seven species) from percent establishment of caterpillars given *Physalis* fruits.

Percent persistence differed by *Physalis* species (Table 7; 2003: $df=12$, $N=336$, Chi-square=97.90, $P < 0.0001$; 2004: $df=2$, $N=65$, Chi-square=6.04, $P = 0.05$). The estimates of persistence for each year were not correlated ($r_s = 0.63$, $P = 0.13$). There was no relationship between the percentage of caterpillars that established on a diet and the percentage of caterpillars that persisted on the diet ($P > 0.30$ for all years).

Relationship between realized and potential host ranges: field versus common garden – The percentage of plants per species infested in the field and in the common garden was positively correlated ($N=7$, $r_s = 0.86$, $P = 0.01$; Fig. 5a). For each species, the number of caterpillars per infested common garden plant was positively correlated to the number of caterpillars per infested plant in the field ($N=7$, $r_s = 0.88$, $P = 0.009$; Fig. 5b) and to the percentage of damaged fruit per infested field plant ($N=7$, $r_s = 0.95$, $P = 0.0008$; Fig. 5c). There was no relationship between the percentage of damaged fruit per infested plant in the common garden and in the field ($N=7$, $r_s = 0.17$, $P = 0.70$; Fig. 5d). The lack of association was due to the large differences between the levels of damage incurred by *P. minuta* plants in the common garden versus plants in the field. When this species was excluded from the analysis, the percentage of fruit per plant with damage in the common garden and the field was positively correlated ($N = 6$, $r_s = 0.94$, $P = 0.005$).

Relationship between realized and potential host ranges: field versus bioassays – The percentage of plants per field site with infestation was correlated to both the percent survivorship ($N = 13$, r

= 0.74, $P = 0.03$; Fig. 6a) and the percent establishment ($N = 13$, $r = 0.79$, $P = 0.001$; Fig. 6b) of caterpillars on fruits of the *Physalis* species in the lab. The number of caterpillars per infested field plant was not correlated with either survivorship ($N = 12$, $r = 0.65$, $P = 0.08$) or percent establishment in the lab assays ($N = 12$, $r = 0.68$, $P = 0.06$). The percent fruit with damage per infested field plant was correlated to the percent survivorship ($N = 13$, $r = 0.86$, $P = 0.002$; Fig. 6e) and the percent establishment ($N = 13$, $r = 0.79$, $P = 0.01$; Fig. 6f) in the no-choice bioassays. Caterpillar persistence on the test diets in the laboratory assays was not correlated with any of the measures of *H. subflexa*'s realized host use in the field ($P > 0.15$ in all tests).

Relationship between realized and potential host ranges: common garden versus bioassays – The percentage of caterpillars that established feeding on fruits of a species was positively correlated with the number of caterpillars per infested plant in the common garden ($r_s = 0.85$, $P = 0.01$, without Bonferroni correction). There was no relationship between percent survivorship or percent persistence in the bioassays and host use in the common garden ($P > 0.10$ in every test).

DISCUSSION

The host range of *H. subflexa* is constrained to a subset of the species in *Physalis* genus. Caterpillars of *H. subflexa* infested plants of 13 of the 17 *Physalis* species that were examined in the field. In the laboratory assays, *H. subflexa* caterpillars had zero percent survivorship on fruits of one *Physalis* species, clearly demonstrating that there are physiological limits to *H. subflexa*'s ability to feed on species in the genus *Physalis*. Caterpillars were able to survive on fruits of some species that *H. subflexa* did not infest in the field. Although *H. subflexa*'s realized host range in the field was smaller than its potential host range as measured in the laboratory, the two were correlated. Likewise, *H. subflexa* infestation in the field tended to be correlated with infestation in the common garden.

Differences among the thirteen species that made up *H. subflexa*'s realized host range in the percentage of plants per field sites with infestation were not as large as the effect of date or the species by date interaction. Some species were more highly infested at high elevation sites and other at low elevation sites. Infestation differed by species in the common garden. The *Physalis* species that were evaluated in the bioassays were not equally suitable hosts of *H. subflexa*. Although *H. subflexa*'s potential host range as measured in the bioassays included species that it did not infest in the field (eg. *P. leptophylla*, *P. lignescens*, and *P. solanaceous*), survival on fruits of these plants in the laboratory assays did not differ from survivorship on fruits of the negative control *N. physalodes*. Agreement between patterns of host use in the field, the

common garden, and the laboratory indicates that host suitability is an important determinant of *H. subflexa* host use. Other studies have found that the herbivore host range is more aligned with plant chemistry (a correlate of suitability) than to the plant species' phylogenetic relationships (Becerra 1997, Wahlberg 2001) or biogeography (Becerra and Venable 1999).

Heliothis subflexa's realized host use was more closely related to the caterpillars' ability to establish on fruits of a species than to the caterpillars ability to persist on fruits of the species. Because the majority of caterpillar mortality happened within the first 96 hours, and there was significant relationship between overall survivorship and infestation in the field, these findings suggest that the chronic effects of factors such as nutrient deficiencies had less of an effect on suitability than acute effects of deterrents or toxins. Failure to establish on a species' fruits may indicate that *H. subflexa* caterpillars do not recognize some fruits as potential hosts, are deterred from feeding, or are prevented from feeding (eg. the viscous substances or the waxes on surface of the fruits of some species may be toxic). The effects of toxins can be particularly acute for first instar larvae (Zalucki *et al.* 2002). Both *Physalis* species and fruit age affect the concentrations of essential fatty acids (Bateman *et al. in prep*) and secondary metabolites (Baumann and Meier 1993). The nutrient composition of mature fruits is notoriously unbalanced (Herrera 1982), and nutrient deficiencies may act synergistically with toxicity (Cornell and Hawkins 2003). These bioassays were carried out without reference to fruit age. Thus, it is impossible to completely dismiss the effects of nutrient content with certainty.

There is little direct evidence for the influence of ecological factors such as predators and competitors. Overall, out of 855 caterpillars collected in the field and in the common gardens in Mexico, only 4 were parasitized (Bateman *et al. in preparation*). All of these caterpillars were collected from plants either in agriculture or in a common garden. (Out of 282 caterpillars collected in common gardens in North Carolina, 19 were parasitized.) Likewise, we rarely observed other frugivores on the field *Physalis* plants, and we never encountered other frugivores feeding on the plants in the common garden.

In addition to plant suitability, present day interactions between *H. subflexa* and the *Physalis* genus appear to be influenced by contemporary biogeographical factors. *Heliothis subflexa* was pervasive at the field sites in West-Central Mexico: it occurred in all ecoregions and across a wide elevation gradient, 15 of the 17 *Physalis* species co-occurred with *H. subflexa* at one or more sites, and 85.5% of the sampled plants were growing at locations where *H. subflexa* was present. The presence of key host species appears to be an important determinant of the presence of *H. subflexa*, but there is no clear geographic pattern explaining why *H. subflexa* was absent from certain sites. Similar results were found in a study on the geographic distributions of

butterflies and their host plants in the United Kingdom: butterfly species often occupied only a small percentage of the sites where their host plants were present, they tended to be more closely associated with the most widely distributed species in their host taxa, and they occurred disproportionately in areas of high food plant species' richness (Quinn *et al.* 1998).

The contrast between *Physalis* species in clusters A and B, relative to the impact of elevation on infestation suggests that elevation differentially affects the attractiveness and/or suitability of *Physalis* species in different ways. Differences may be associated with the affects of unaccounted for shifts in *Physalis* species' numeric representation at high versus low elevations on oviposition preference. The elevational differences in host use could also be related to *H. subflexa* population isolation and differentiation. The common garden took place at a high elevation site, and the insects used in the bioassays were collected from a high elevation population. If lowland and highland populations have differentiated, the outcomes of bioassays using *H. subflexa* colonies of lowland origin may not be the same as those reported here. Studies of population structure, additional bioassays using separate colonies from high and low sites, and reciprocal transplant studies could help to separate factors associated with environmental factors versus population differentiation.

In conclusion, despite very strong effects of species by date and species by location interactions on field infestation and damage, the overall pattern of *H. subflexa* realized host range is correlated with host use in a common garden and with fruit suitability for larval survival as measured in the laboratory.

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TABLE 1. Description of the 17 *Physalis* species examined in this study. The species that were examined in the field, common garden, and the bioassays are denoted with an “X”.

<i>Physalis</i> species	Life span ¹	Pubescence ¹	Geographic range ^{1,2}	Ecoregion ^{1,3}	Altitudinal gradient (max-min) ^{1,3}	Field	Common garden	Bioassays
<i>P. ampla</i>	Annual	Dense, nonglandular pubescence	Mexico	Trans-Mexican Volcanic Belt and Sierra Madres conifer-oak forests, Southern Mexican dry forests	690 m to 1,934 m	X	X	X
<i>P. angulata</i>	Annual	Nearly glabrous	North and South America; introduced Old World Tropics	Central Mexican matorral, Southern Mexican dry forest, Trans-Mexican Volcanic Belt and Sierra Madre conifer-oak forests	890 m to 2,415 m	X	X	X
<i>P. cinerascens</i>	Perennial	Dense, nonglandular pubescence	US and Mexico	Central Mexican matorral	.	X	-	-
<i>P. cordata</i>	Annual	Nearly glabrous	North and South America; introduced Old World Tropics	Trans-Mexican Volcanic Belt conifer-oak forest, Southern Mexican dry forest, South Pacific Coast mangrove & palm	2 m to 1,850 m	X	X	X
<i>P. glutinosa</i>	Perennial	Dense, glandular pubescence	Mexico	Central Mexican Matorral	.	X	-	-
<i>P. hastatula</i>	Perennial	Nearly glabrous	Mexico	Central Mexican Matorral	1,931 m to 2,216 m	X	-	-
<i>P. lagascae</i>	Perennial	Dense, glandular pubescence	Mesoamerica; likely introduced to Old World	Central Mexican matorral, Southern Mexican dry forest, South Pacific Coast mangrove, Trans-Mexican Volcanic Belt and Sierra Madre conifer-oak forests	13 m to 1,934 m	X	X	X
<i>P. leptophylla</i>	Annual	Dense, glandular pubescence	Mexico	Southern Mexican dry forest	35 m to 70 m	X	-	X
<i>P. lignescens</i>	Perennial	Dense, nonglandular pubescence	Jalisco endemic	Trans-Mexican Volcanic Belt conifer-oak forest	.	X	-	X
<i>P. minuta</i>	Annual	Dense, nonglandular pubescence	Mesoamerica	Southern Mexican dry forest	2 m to 29 m	X	X	X
<i>P. nicandroides</i>	Annual	Dense, glandular pubescence	Mesoamerica	Central Mexican matorral, Southern Mexican dry forest, Trans-Mexican Volcanic Belt and Sierra Madre conifer-oak forests	890 m to 2,415 m	X	X	X
<i>P. orizabe</i>	Perennial	Dense, nonglandular pubescence	Trans-Mexican Volcanic Belt	Trans-Mexican Volcanic Belt conifer-oak forest	1554 m to 2,397 m	X	-	-
<i>P. patula</i>	Annual	Dense, glandular pubescence	Mexico	Central Mexican matorral	2,173 m to 2,216 m	X	-	X
<i>P. philadelphica</i>	Annual	Nearly glabrous	Mexico, introduced elsewhere as an agricultural crop	Central Mexican matorral, Southern Mexican dry forest, Meosamerican and Sierra Marde conifer-oak forests	10 m to 2,415 m	X	X	X
<i>P. pruinosa</i>	Annual	Nearly glabrous	North and South America	Southern Mexican dry forest, Trans-Mexican Volcanic Belt and Sierra Madre conifer-oak forests	35 m to 1,850 m	X	-	X
<i>P. pubescens</i>	Annual	Dense, nonglandular pubescence	North and South America; introduced Old World Tropics	Central Mexican matorral, Trans-Mexican Volcanic Belt and Sierra Madre conifer-oak forests, Southern Mexican dry forests	35 m to 1,960 m	X	X	X
<i>P. solanaceus</i>	Annual	Nearly glabrous	Southern US and Mexico	Central Mexican matorral	1,000 m to 2,415 m	X	-	X

¹. Vargas *et al.* 2003, ². Martínez 1993, ³. Refer Chapter 3 of dissertation.

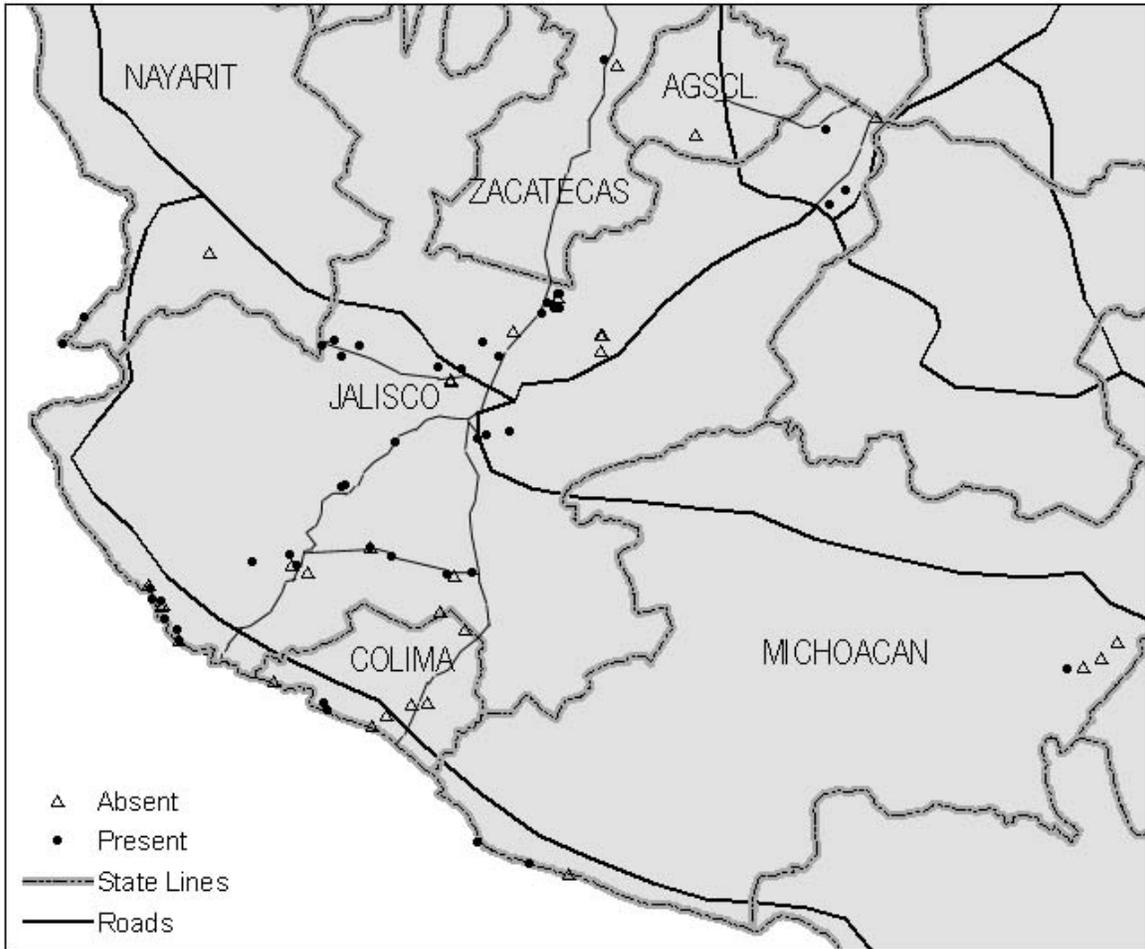


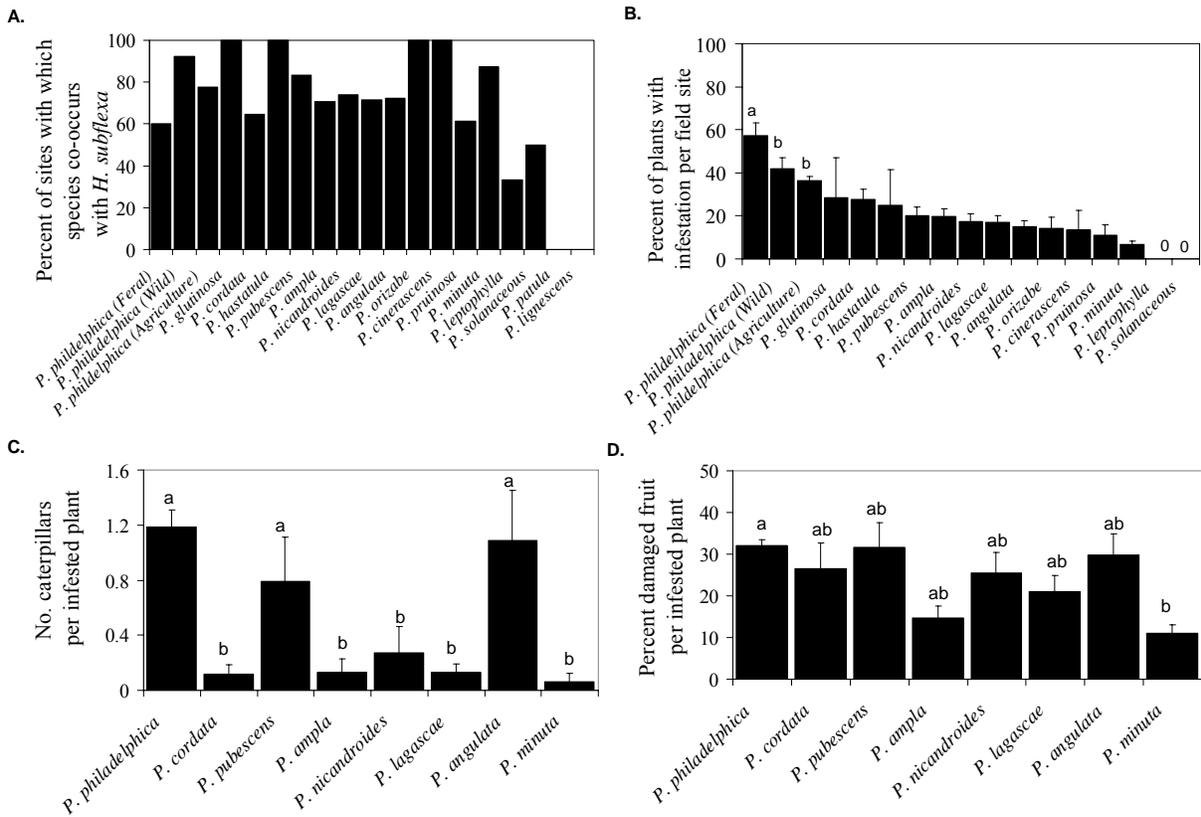
FIG. 1. The 76 field sites where we assessed *H. subflexa*'s infestation to fruiting plants of 17 *Physalis* species. Open triangles indicate that *H. subflexa* was not detected at a field site. Closed circles indicate that *H. subflexa* was present at the site.

TABLE 2. Time period of data collection in the field in each year of the study.

Year	Start	End
2002	September 26	November 17
2003	August 6	November 22
2004	August 9	November 29

TABLE 3. Description of the *H. subflexa* laboratory colonies that were used in the bioassays and the conditions under which they were maintained.

Colony origin	Location of assays	Dates of experiments	Colony diet	Colony conditions	
				Light-dark cycle	Temperature
MEXICO, Jalisco, Municipality of Ixtlahuacán del Río, towns of Las Cruces, Coyotillo, Jaguecito. On fruits of <i>P. philadelphica</i> . July 28, 2003.	CUCBA	9/8/2003 to 12/26/2003	Corn-soy blend and Southland Products artificial diet	12-hr/12-hr	Average=20.13°C (Minimum: 12.5°C, Maximum: 27.2°C)
MEXICO, Jalisco, Municipality of Ixtlahuacán del Río, town of Coyotillo. On fruits of <i>P. philadelphica</i> . August 3, 2004 .	Estación de Biología Chamela (UNAM), Chamela, Jalisco, Mexico	9/30/2004 to 11/14/2004	Southland Products artificial diet	12-hr/12-hr	Average=27.09 ±0.01°C (Minimum=22.9°C, Maximum=30.71°C)



Physalis species

FIG. 2. *Heliothis subflexa*'s associations with plants of 17 *Physalis* species at field sites in West-Central Mexico. A plant was considered to be infested based on the presence of *H. subflexa* caterpillars and/or *H. subflexa*'s stereotypical damage. (A.) The percentage of sites at which a species co-occurred with *H. subflexa*. (B.) Percentage of plants with infestation per site where *H. subflexa* was present. (C.) Mean number of caterpillars per infested plant. (D.) Percentage of fruit with damage per infested plant ($N > 10$).

TABLE 4. Results of logistic regression on the percentage of plants with *H. subflexa* infestation at field sites that we visited multiple times over the course of the season.

	Num df	Den df	F	P
<i>Physalis</i> species	7	128	2.64	0.01
Location	17	128	5.83	<0.0001
Date	1	128	3.67	0.06
<i>Physalis</i> species x Date	8	128	1.94	0.05

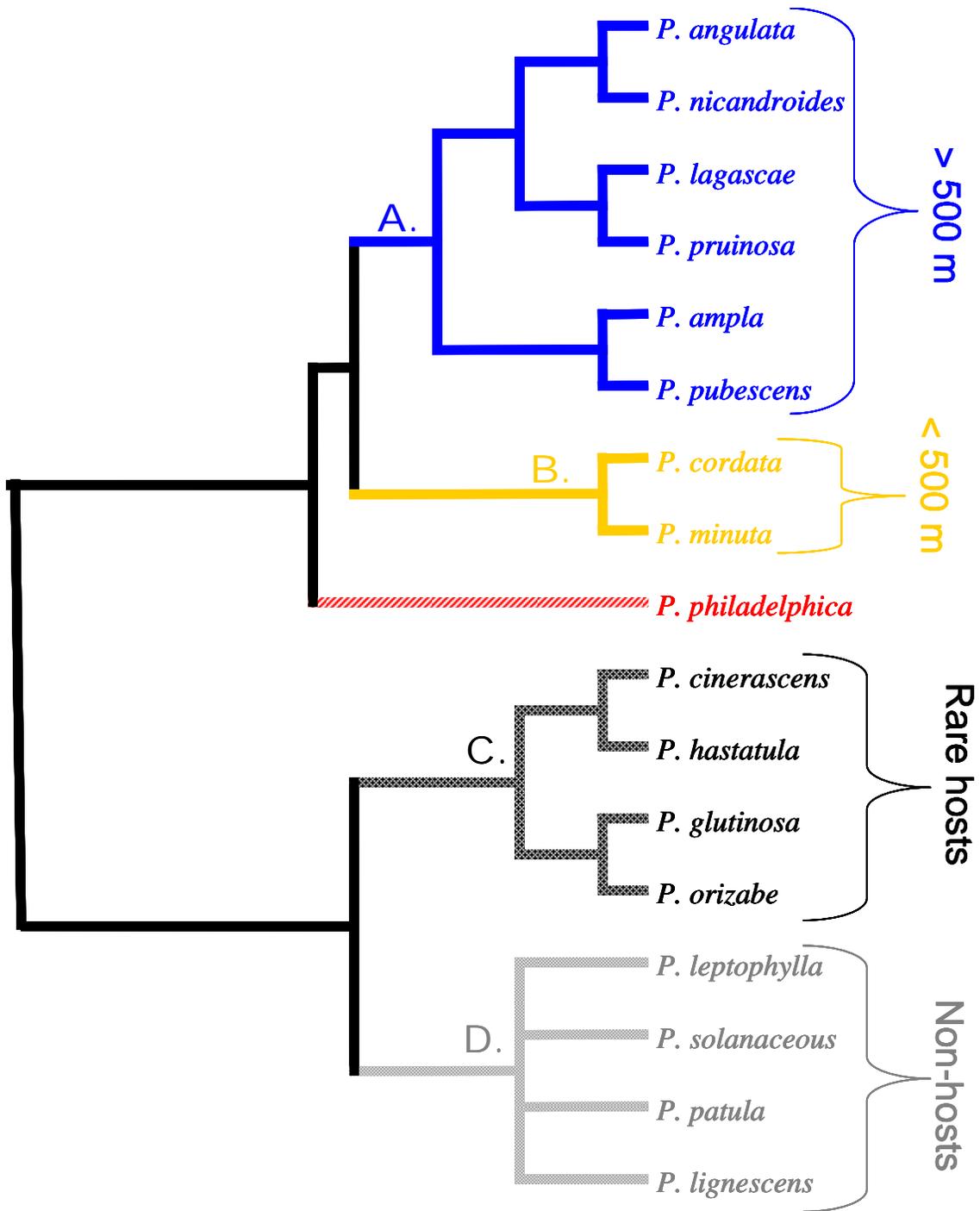


FIG. 3. Dendrogram of *H. subflexa*'s host use based on the similarity of geographical patterns of *H. subflexa* infestation. A. *Physalis* species that were typically infested at sites above 500 m in elevation, B. *Physalis* species that were infested at sites below 500 m in elevation, C. infrequent hosts that were only infested when they co-occurred with plants in Cluster A, and D. species that were not part of *H. subflexa*'s realized host range.

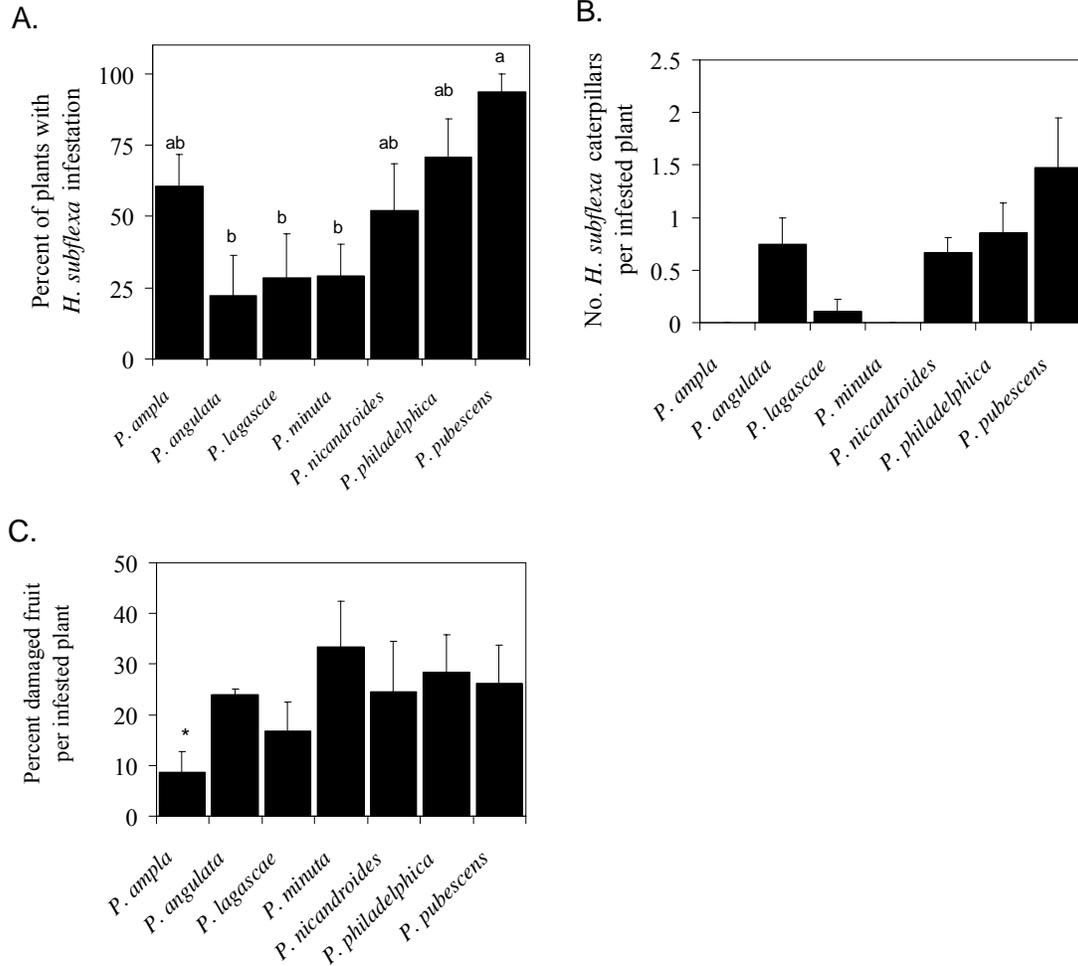
TABLE 5. Results of paired *t*-tests on the percentage of infested field plants for two different sets of *Physalis* species that frequently co-occur at the field sites both in the wild and in agriculture (* *P* < 0.05 with Bonferroni correction for tests in each set of contrasts [*i.e.* 8 comparisons among Cluster A plants on tomatillo farms, 20 among Cluster A plants in wild populations, and 3 comparisons among Cluster B plants).

Cluster A, Tomatillo farms with wild plants

	<i>P. ampla</i>	<i>P. angulata</i>	<i>P. lagascae</i>	<i>P. nicandroides</i>	<i>P. philadelphica</i>	<i>P. pruinosa</i>	<i>P. pubescens</i>
Cluster A, Wild populations	-	-	t = 6.25	t = -3.36	-	t = 0.21	<i>P. ampla</i>
	t = -1.22	-	-	t = -16.58 *	-	-	<i>P. angulata</i>
	t = 0.36	t = -0.25	-	-	-	-	<i>P. lagascae</i>
	t = 0.19	t = 0.27	t = -2.77	t = -25.78 *	-	t = -1.0	<i>P. nicandroides</i>
	t = 1.00	t = -1.15	t = 0.55	t = 1.11	-	t = 4.49	<i>P. philadelphica</i>
	t = 0.009	t = -0.49	t = 4.00	t = 0.35	t = -1.27	-	<i>P. pruinosa</i>
	t = 2.27	t = 1.54	t = -0.67	t = -1.68	t = 1.28	-	<i>P. pubescens</i>

Cluster B

	<i>P. minuta</i>	<i>P. philadelphica</i> (Agriculture)	<i>P. philadelphica</i> (Wild)	<i>P. cordata</i>
	t = 1.12	t = -0.93	t = -1.86	



Physalis species

FIG 4. *Heliiothis subflexa* infestation to plants of seven *Physalis* species grown in a common garden in Jalisco, Mexico. A plant was considered to be infested based on the presence of *H. subflexa* caterpillars and/or *H. subflexa*'s stereotypical damage. (A.) The percentage of common garden plants of each species with *H. subflexa* infestation. Histogram bars with the same letter do not differ significantly between treatments at $P < 0.05$. (B.) The mean number of caterpillars per infested plant. (C.) The percentage of damaged fruit per infested plant. Asterisk denotes significant difference at $P < 0.05$. Error bars represent +1 SE.

TABLE 6. Results of analysis of variance on the percentage of *H. subflexa* infestation to common garden plants of seven *Physalis* species.

	df	MS	F	P
A) Whole model				
Model	52	0.38	2.39	0.0003
Error	73	0.16		
Corrected total	1981			
B) Model components				
<i>Physalis</i> species	6	1.01	3.84	0.004
Block	7	0.27	1.02	0.43
<i>Physalis</i> species x Block	39	0.28	1.75	0.02

TABLE 7. *Heliothis subflexa* caterpillar performance in no-choice bioassays of feeding and survival on 13 *Physalis* species, artificial diet, and three non-host species. N for each test diet is listed in parentheses. For test diets that were evaluated in the same year, test diets with the same letter do not differ significantly at $P < 0.05$ in multiple comparisons among species with an adjustment for multiple tests using the Bonferonni method.

	Overall Percent Survivorship	Percent Establishment	Percent Persistence
2003:			
<u>Control diets:</u>			
Artificial diet	73.73 (83) a	95.18 (83) a	77.63 (76) ab
<i>J. procumbens</i>	0.00 (75)	0.00 (75)	0.00 (75)
<i>N. physalodes</i>	4.17 (72) e	56.76 (74) bcd	7.5 (40) d
<i>S. americanum</i>	0.00 (75)	0.00 (75)	0.00 (75)
<u>Physalis species:</u>			
<i>P. ampla</i>	29.58 (71) bcd	40.54 (74) def	77.78 (27) ab
<i>P. angulata</i>	45.95 (74) b	52.00 (75) cde	89.47 (38) a
<i>P. cordata</i>	32.36 (62) bcd	61.63 (86) bcd	68.97 (29) abc
<i>P. lagascae</i>	18.92 (74) cde	21.33 (75) f	93.33 (15)
<i>P. leptophylla</i>	2.08 (48) e	16.67 (48) fg	12.50 (8)
<i>P. lignescens</i>	5.71 (70) e	42.50 (80) def	16.67 (24) d
<i>P. minuta</i>	17.14 (70) cde	28.95 (76) ef	75.00 (16)
<i>P. nicandroides</i>	13.16 (76) de	51.72 (87) cde	29.41 (34) d
<i>P. patula</i>	0.00 (72)	21.62 (74) f	0.00 (14)
<i>P. philadelphica</i>	30.3 (66) bcd	75.00 (76) b	42.55 (39) bcd
<i>P. pruinosa</i>	7.58 (66) e	32.05 (78) ef	38.46 (13)
<i>P. pubescens</i>	33.8 (71) bc	69.33 (75) bc	50.00 (48) bcd
<i>P. solanaceous</i>	4.88 (82) e	28.92 (83) f	17.39 (23) d
2004:	335	359	219
Artificial diet	31.52 (92) a	100.00 (92) a	31.52 (92) ab
<i>P. ampla</i>	4.65 (43) b	55.1 (49) b	9.52 (21) b
<i>P. angulata</i>	16.67 (36) ab	67.50 (40) b ±	26.09 (23) ab
<i>P. lagascae</i>	0.00 (30)	16.67 (30) c	0.00 (5)
<i>P. minuta</i>	23.68 (38) a	57.5 (40) b	42.86 (21) a
<i>P. philadelphica</i>	0.00 (30)	76.32 (38) b	0.00 (21)
<i>P. pruinosa</i>	0.00 (39)	52.50 (40) b	0.00 (20)
<i>P. solanaceous</i>	0.00 (27)	63.33 (30) b	0.00 (16)
Total (N):	1306	1427	560

POTENTIAL HOST RANGE

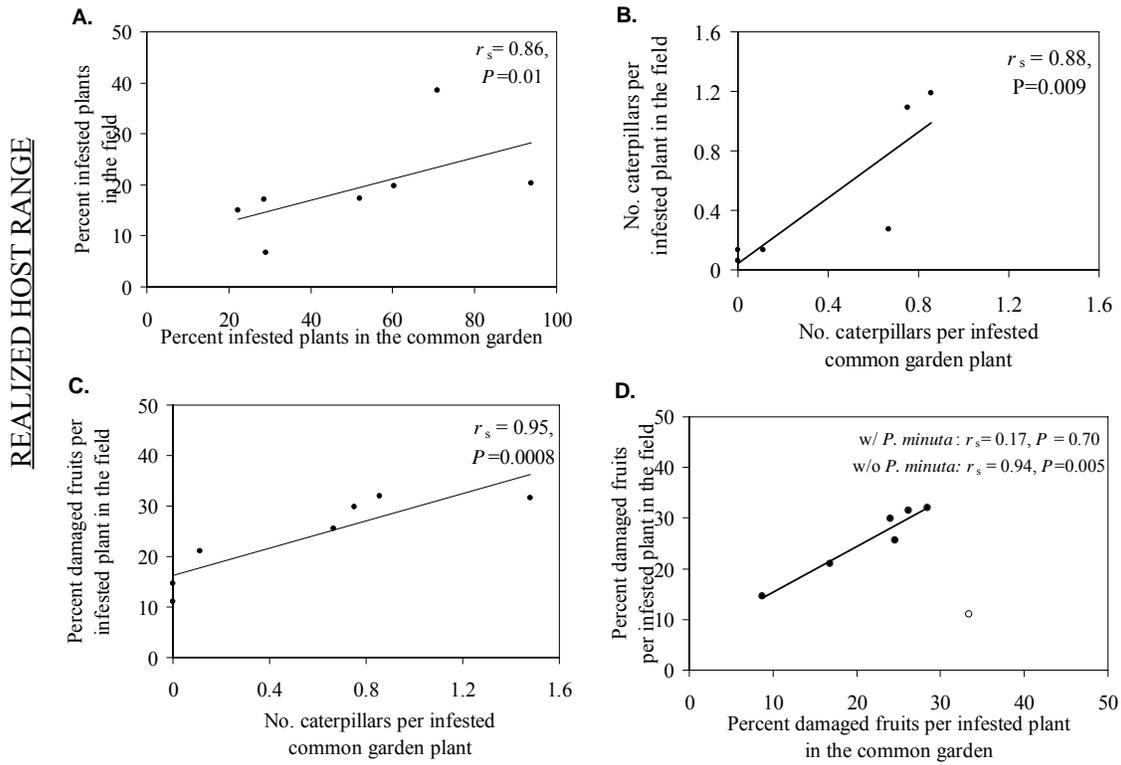


FIG. 5. Correlations between potential host range as measured through observations in a common garden and realized host use as measured through observations at sites in the field.

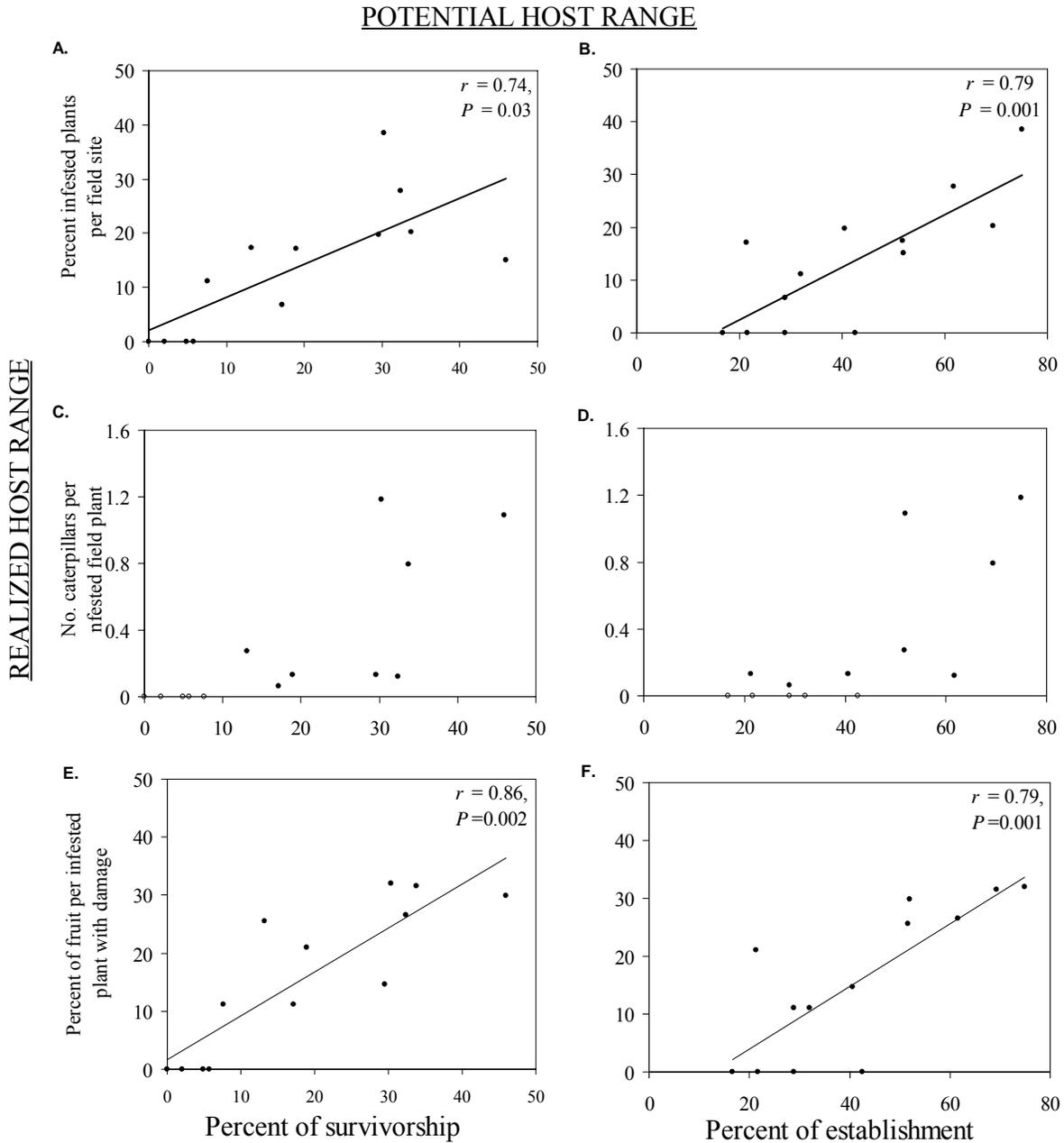


FIG. 6. Correlations between *H. subflexa*'s realized host range as measured by the frequency and intensity of infestation to field plants (rows) and its potential host range as measured by no-choice bioassays (Columns). Survivorship is the percentage of caterpillars that survived from neonate to pupation, and establishment is the percentage of caterpillars that initiated feeding on a diet.

**3. BIOGEOGRAPHICAL PATTERNS OF *PHYSALIS* (SOLANACEAE) DISTRIBUTION
IN WEST-CENTRAL MEXICO**

ABSTRACT

Aim Mexico as a whole has a distinctive flora, both in terms of species richness and endemism. On a regional scale within Mexico, levels of overall plant biodiversity vary considerably. The abundance of individual species differs at regional as well as local spatial scales. The aim of this study was to assess patterns in the geographic distribution of species in the genus *Physalis* L. (Solanaceae) and to compare trends in biogeography of this genus with that of the aggregate of Mexican flora.

Location Between 18° to 23° North and 100° to 105° West in the West-Central Mexican states of Aguascalientes, Guerrero, Jalisco, Michoacán, Nayarit, and Zacatecas.

Methods We assessed the frequency with which plants of 26 different *Physalis* species occurred at 118 field sites in West-Central Mexico. We measured the density of *Physalis* plants occurring at 11 locations. We evaluated the realized niche of the 26 species in terms of the number of ecoregions and the altitudinal gradient across which they occurred. We tested for relationship between a species' density within a site, the frequency with which the species was present at the 118 study sites, the size of its overall geographic range, and its realized niche breadth. To describe the biogeography of the genus as a whole, we calculated species richness and an endemism score for each of the 118 sites.

Results The species that inhabited the greatest proportion of the sampled sites also tended to have larger geographic ranges and broader realized niches. We identified five different groups of species that tended to co-occur. For the genus as a whole, the mean species richness of the sites did not differ by ecoregion but did increase with elevation. More endemic species were present in the Central Mexican Matorral and the mountains of the Trans-Mexican Volcanic Belt.

Main Conclusions In West-Central Mexico, *Physalis* species with the largest overall geographic ranges also tended to occur with the greatest frequency and across the broadest assortment of habitats. High levels of endemism were associated with the ecological islands of the Central Mexican Matorral and the Trans-Mexican Volcanic Belt. These are regions with high levels of endemism for other Mexican floristic groups. Findings of this study suggest two avenues for future research. Phylogenetic studies

could be used to elucidate species' relationships and to test whether narrowly endemic species are relatively new radiations.

Keywords: geographic range size, niche breadth, endemism, species richness, *Physalis*, Mexico

INTRODUCTION

While Mexico accounts for only 1.5% of the planet's terrestrial land mass, approximately 10-12% of the world's species occur there (Toledo and Ordóñez 1993, Bobbink and Heil 2003, Serna *et al.* 2004). The whole range of major plant growth forms is present, including some that are unique to Mexico (Rzedowski 1993). A high percentage of the flora and fauna occurring in Mexico are endemic (Rzedowski 1993, Villaseñor 1998, Lira *et al.* 2002, Sechrest *et al.* 2002, Dávila *et al.* 2004). Twelve of the world's plant genera are endemic to Mexico (Ramamoorthy and Lorence 1987), and 52% of the plant species that occur in Mexico are not found elsewhere (Rzedowski 1993). Overall, Mexico's floristic wealth can be attributed to the country's topography, history of climate change, present day environmental conditions, and unique position at the juncture of Nearctic and Neotropical realms (Bobbink and Heil 2003). Three major tectonic plates meet in Mexico (North American, Pacific, and Cocos plates). An extension of the North American plate forms the Mexican Central Plateau. This plateau is bordered by the Sierra Madres, and by the Trans-Mexican Volcanic Belt in the south (Bobbink and Heil 2003). These topographic features and their associated climatic conditions subdivide the country into a mosaic of distinct ecoregions, each with its own characteristic flora (Rzedowski and McVaugh 1966, Rzedowski 1978, Olson and Dinerstein 1998).

Floristic studies of large samples of plant taxa indicate that Mexican ecoregions differ in terms of species richness and endemism (Villaseñor 1990, Rzedowski 1993, Aljos 1996, Lira *et al.* 2002, Delgadillo *et al.* 2003, Dávila *et al.* 2004). Species richness is highest in the south in Chiapas and Oaxaca and along two belts that extend north, one towards Veracruz in the east and the other towards Sinaloa and Durango in the west (Rzedowski 1993). Overall, a higher percentage of species (24%) occur in conifer and oak forests, but on a per area basis cloud forests are the most species rich (Rzedowski 1993). The geographic distribution of centers of endemism differs from that of the zones of species richness. Endemism is highest on "ecological islands" such as the high peaks of the Trans-Mexican Volcanic Belt or in isolated xeric environments such as the Central Mexican Matorral (Rzedowski 1993, Bobbink and Heil 2003). This matches expectations in that population isolation favors lineage splitting and the evolution of new species.

The spatial distribution of an individual species can be defined at multiple scales (*e.g.*: the size of the species' overall geographic range, the proportion of sites occupied by the species within its geographic range, and the species' abundance at a particular site). In comparative studies of ecologically similar co-occurring species, the measures of distribution at the different scales tended to be positively correlated (Brown 1984). More widespread species tend to be common and abundant within their geographic ranges. A species' spatial distribution may be determined by the range of environmental conditions that it is able to tolerate, or its "niche breadth" (Gaston and Spicer 2001). Seed dispersal (Venable and Brown 1988) and competitive ability (Walck *et al.* 2001) have also been shown to be related to species' geographic distributions.

One group of plants with a Mexican center of diversity is the genus *Physalis* (Solanaceae) (Waterfall 1967, Martínez 1998, Vargas 1998, Whitson and Manos 2005). Seventy of the approximately 90 *Physalis* species (estimates range from 75 to 140) are endemic to Mexico (Waterfall 1967, Martínez 1993, Vargas *et al.* 2003, Whitson and Manos 2005). Six species are found only in Central America, and five species are found exclusively in the United States. Only one species (*P. alkekengi*) is native to the Old World, but some species have been secondarily introduced to tropical Africa, Asia, and Australia (Martínez 1993, Vargas *et al.* 2003). The geographic distribution of the individual species varies from cosmopolitan to narrowly endemic (Waterfall 1958, 1967; Martínez 1993). In most parts of the world, *Physalis* tends to be rare, but in Mexico *Physalis* patches are relatively common in disturbed habitats. Members of the *Physalis* genus can occur in all but the most extreme environments, but the species' identity changes from location to location (Vargas 1998, Vargas *et al.* 2003). While taxonomic publications document specific field sites where *Physalis* species occur (Waterfall 1967, Nee 1986, Martínez 1993, Martínez 1998, Vargas 1998, Vargas *et al.* 1998, Estrada and Martínez 1999, Vargas *et al.* 2003, Sullivan 2004), integration of information on the distributions of the individual species and the genus as a whole is lacking. Because herbarium specimens do not always include geographic coordinates, the ability to use them in spatially explicit comparisons is limited. Likewise, pressed specimens may not give an accurate picture of which species co-occur in the field since the notes that accompany voucher specimens frequently do not state whether conspecifics were present. Similarly, the collection of specimens of two different species from the same general area but on separate occasions does not necessarily mean that the two species ever co-occur. Field data can be used to attain a better understanding of the distribution and co-occurrence of *Physalis* species. Quantitative characterization of *Physalis* biogeography may provide insight into the ecology and evolution of this group of plants.

The goal of the current study was to identify patterns of *Physalis* species distributions and to compare the biogeography of this genus to that of other Mexican taxa. More than one third (39 out of 90) of the known *Physalis* species grow in West-Central Mexico, making *Physalis* species richness higher in this region than in any other part of Mexico (Vargas 1998, Vargas *et al.* 1998, Vargas *et al.* 2003). Over a period of three years, we surveyed 118 locations in West-Central Mexico. For the individual *Physalis* species, we assessed the relationship between the size of the species' overall geographic range, the frequency with which a species occurred at the sampled sites in West-Central Mexico, abundance at a site, and niche breadth. To identify which *Physalis* species tended to co-occur, we constructed a dendrogram using Jaccard similarity indices and UPGMA (Unweighted Pair Group Method with Arithmetic mean). For the genus as a whole, we 1) identified localities in the study area with elevated species richness and endemism, 2) evaluated the degree of overlap of the centers of species richness and endemism, 3) tested whether levels of species richness or endemism differed by habitat, and 4) evaluated whether centers of endemism were associated with "ecological islands". Finally, we assessed whether trends in *Physalis* biogeography mirrored patterns observed for the aggregate of Mexican flora.

METHODS

The *Physalis* genus

Within the family Solanaceae, the *Physalis* genus is placed in the subfamily Solanoideae along with comparatively well-studied genera like *Capsicum*, *Datura*, and *Solanum* (Olmstead *et al.* 1999). The *Physalis* genus is in the tribe *Physaleae* and the subtribe *Physalinae*. While the genus as a whole is paraphyletic, the morphologically typical *Physalis* species form a monophyletic clade (Whitson and Manos 2005). The base of the morphologically typical *Physalis* is composed of mostly annual species with a Mexican center of origin. Two separate perennial clades are located more distally on the tree. Species in one of the perennial clades are Mexican endemics, and the species in the other perennial clade are a mix of both US and Mexican species. A two-gene molecular phylogeny has clarified the species' relationships of the clade of perennials that extends into the US, but relationships among many of the Mexican endemics have yet to be resolved (Martínez 1993, Whitson and Manos 2005).

Description of the study area

Over the course of three years we examined 118 locations between 18° – 23° North and 100° – 105° West in the Mexican states of Aguascalientes, Colima, Jalisco, Guerrero, Michoacán, and Zacatecas (Figure 1, Appendix A). Important natural features in this region include the narrow

band of the Pacific Coastal lowlands, the Occidental Sierra Madre, the Trans-Mexican Volcanic Belt, the Balsas Depression, and the Central Mexican Plateau (Table 1; Vargas 1998). The study area includes portions of six ecoregions: the Central Mexican Matorral, the Occidental Sierra Madre Conifer-Oak forest, the Trans-Mexican Volcanic Belt Conifer-Oak forest, the Southern Mexican Dry Forest, and on the coast small patches of South Pacific Coast Mangroves and Dune (Rzedowski and McVaugh 1966, Rzedowski 1978, Olson and Dinerstein 1998, Vargas 1998, Challenger 1998, Olson *et al.* 2001). These ecoregions each have their own characteristic flora. Within the study area as a whole, elevation ranges from sea level to 4,300 meters. The study region includes some of the areas of species richness and endemism that have been previously identified for the aggregate of Mexican flora (Rzedowski 1993, Aljos 1996, Lira *et al.* 2002, Delgadillo 2003). The western belt of plant species richness crosses the study area (Bobbink and Heil 2003). For several taxonomic groups, elevated levels of species endemism are found in the xeric environments of the Central Mexican Matorral and in association with mountains in the Trans-Mexican Volcanic Belt (Halffter 1988, Delgadillo *et al.* 2003, Dávila *et al.* 2004).

Data collection in the field

We sought to examine multiple field sites in each of the major ecoregions and to take data on as many of the 39 species as possible. In general, we examined accessible sites with moderate disturbance such as roadsides, pastures, cornfields, arroyos, and trails. We repeatedly examined locations spaced along seven routes: 1.) from Guadalajara north to Zacatecas, 2.) from Guadalajara northeast towards San Luis Potosi, 3.) from Guadalajara west to the coast via Tepic, 4.) from Guadalajara southwest to the coast via Autlán, 5.) from Autlán to Ciudad Guzmán, 6.) from Guadalajara south to the coast via Colima, and 7.) along the coast from Bahía Maruata to Puerto Vallarta. One time only, we also examined plant populations between Morelia and Taxco. These routes correspond to most of the major roads, some secondary roads, and some unimproved dirt roads in Jalisco and Colima as well as portions of the neighboring states. They traverse a comprehensive cross-section of the habitats present in West-Central Mexico. To maximize the number of observations of sites where little known species were potentially present, the selection of the general area of 55 sites was guided by taxonomic keys (Vargas 1998, 2003), consultation of herbarium specimens (Instituto Botánica de la Universidad de Guadalajara, Guadalajara, Jalisco), and discussions with botanists and tomatillo farmers.

Using GPS units (Garmin Etrex Summit), we acquired the geographic coordinates and elevation of each location that we examined. At each site, we searched a minimum 200 m² area. At 16 locations, we never found any *Physalis* plants (after searching for a minimum of 15

minutes). Prior records of *Physalis* populations exist for 15 of the 102 positive sites. Observations at the remaining 83 sites were new registers. Voucher specimens of the *Physalis* plants were deposited at the Instituto Botánica de la Universidad de Guadalajara and the Estación de Biología Chamela, UNAM.

The date range of the study covered the rainy season and the beginning of the dry season, the time period in which *Physalis* is most abundant. Specifically, we examined the field sites from July to the end of November (2002: September 26th to November 17th; 2003: July 7th to November 22nd; and 2004: August 4th to November 29th). During the three-year period, we spent a total of 99 days actively searching for field populations of *Physalis*. On average, we examined 2.5 locations per day. Seventy-six of the locations were examined on one occasion only, and forty of the sites were observed on multiple occasions over the course of the growing season (two to eight times). Some sites were visited on multiple occasions to assess whether the number of species encountered would increase with subsequent observations. Upon returning to a site after the initial survey, in 68.2% of the visits we found exactly the same set of species, in 10% of the visits we found between one and two additional species, and in 21.8% of the visits we found one to three fewer species. Based on the similarities in the set of species that we encountered during each visit, the number of trips to a site should not have substantially biased the number of species observed for that site. Much of the change over time in the species composition of the sites can be explained by species-specific seasonal phenology (see Chapter 4).

Assessment of *Physalis* biogeography

To determine the sizes of the individual species' geographic ranges, we referred to the literature (Waterfall 1967, Nee 1986, Martínez 1993, Martínez 1998, Vargas 1998, Vargas *et al.* 1998, Vargas *et al.* 2003). Based on the species' overall geographic ranges, we assigned the species to the following five categories: cosmopolitan (throughout the Americas and introduced in to the Old World), Mesoamerican (Central Mexico to Central America and the Antilles), US and Mexico, Mexico only, or narrow endemic (only select sites in Jalisco). As a measure of how common the species were within West-Central Mexico, we calculated the frequency with which each species occurred at the 118 sites examined.

At eleven separate locations we measured *Physalis* plant density (Table 2). At each of these eleven locations, we mapped all of the individuals of each species following the method described in Boose *et al.* (1998). Measurement at these sites took place in October, after plant populations were well established but prior to senescence. The eleven field sites where plant density was measured were selected to represent a broad spectrum of the habitats in West-Central

Mexico. They were located in five ecoregions: Central Mexican Matorral (two locations), Occidental Sierra Madre pine-oak forest (one location), Trans-Mexican Volcanic Belt pine-oak forest (five locations), Southern Mexican dry forest (five locations), and South Pacific Coast beaches (three locations). Likewise, the sites bracketed the minimum and maximum elevations of the 118 study sites (sea level to 2,397 m in elevation).

Because of the interaction between trade winds and topography, the climates of sites at the same elevation are not necessarily the same. Consequently, the realized niche breadth of each species was evaluated both in terms of the number of ecoregions and the altitudinal gradient across which a species occurred.

To assess which species co-occurred, we calculated the Jaccard similarity index of the species composition of the sites:

$$\text{Jaccard similarity index} = \frac{a}{a+b+c}$$

where **a** is the number of sites occupied by both species *i* and *j*, **b** is the number of sites occupied only by species *i*, and **c** is the number of sites occupied only by species *j*. We then clustered the species hierarchically using UPGMA (Unweighted Pair Group Method with Arithmetic Mean; Sneath and Snokal 1973). To confirm the robustness of the dendrogram, we also constructed a dendrogram using Euclidean distances and Ward's method (Everitt *et al.* 2001). Distance matrices and the dendrograms were constructed using ADE-4 Software (Thioulouse *et al.* 2001).

To assess the biogeographic diversity patterns of the genus as a whole, we calculated the mean number of species per site in each of the ecoregions. To evaluate geographic patterns in levels of endemism, we calculated an endemism score (ES) for each site (Rebelo 1992):

$$\text{Raw ES} = \sum_i^j \left(\frac{118}{a_i} \right)$$

where *j* is the number of species at each site, *a* is the number of sites containing species *i*, and 118 is the total number of field sites. To correct for species richness, the raw endemism scores for each site were divided by the number of *Physalis* species at each location.

Statistical analyses

Using a general linear model, we tested whether the mean elevation of the sites differed by ecoregion. Fisher's exact test was used to assess whether there was a significant association between the ecoregion with which a site was associated and the presence of *Physalis* species at the site. We tested whether the presence of *Physalis* differed by elevation using logistic linear regression.

A chi-square contingency table was used to test whether the species differed in the frequency with which they were present at the 118 sites. Whenever the overall test for equality of plant species was significant at 0.05, plant species were compared two at a time using either chi-square or Fisher's exact test (whenever sample sizes in 50% of the cells were less than five) with correction for multiple comparisons using the Bonferroni procedure. Only species that occurred at five or more sites were included in this analysis. To test for differences in species' mean plant density per site, we used the Kruskal-Wallis H test. Spearman's Rho was used to test for correlation between the average plant density of a species and the percentage of the study sites at which the species occurred. We evaluated whether the percentage of sites occupied and plant density differed by lifespan (perennial versus annual) using Student's *t*-test.

Using the Kruskal-Wallis H test, we evaluated whether the percentage of sites occupied by a species and/or plant density at the sites differed by the size of the species' geographic range. The tomatillo (*P. philadelphica*) is an agricultural crop and was consequently excluded from these analyses. Kruskal-Wallis H test was used to evaluate whether the gradient in elevation or the number of ecoregions across which a species occurred differed by geographic range size. Pearson's test of correlation was used to assess the relationships between the percentage of sites at which the species occurred, average plant density at a site, the number of ecoregions in which a species occurred, and the altitudinal gradient across which a species was found. The percentage of study sites occupied by a species was arcsine-square root transformed to approximate a normal distribution of occupation values.

Following hierarchical clustering, the Kruskal-Wallis H test was used to assess whether the clusters of co-occurring species differed with respect to the percentage of sites at which the species occurred, the number of ecoregions with which they were associated, or the altitudinal gradient across which they were found.

For the genus as a whole, we tested for correlation between the sites' species richness and endemism scores. We tested whether either the average species richness or the endemism score of the sites differed by ecoregion using Kruskal-Wallis H test. We also evaluated whether species richness or endemism was correlated with elevation. Since other studies of Mexican flora have shown that endemics are often associated with mountain peaks, we used regression to test whether endemism was positively correlated with elevation.

All statistical analyses were conducted using SAS software (SAS 8.2 for Windows, SAS Institute, 2001). In all tests, the significance level was set at $\alpha=0.05$, and ± 1 standard error (SE) values are presented for estimated mean values. Following significant Kruskal-Wallis H tests,

posterior tests of more specific contrasts were performed using Mann-Whitney U tests. Multiple comparisons were Bonferonni corrected.

RESULTS

Proportion of field sites where the *Physalis* genus was present

The sites occurred between sea level and 2,415 m in elevation. The ecoregions differed significantly in elevation ($F=43.81$, $df=4$, 107 ; $P < 0.0001$; Fig. 2a). *Physalis* populations occurred in all of the major ecoregions in the study area, and ecoregions did not differ when compared based on the percentage of sites where at least one representative of the *Physalis* genus was present (Fisher's exact test, $P = 0.71$; Fig. 2b). However the percentage of sites occupied by *Physalis* differed significantly by elevation (Logistic regression: Wald Chi-square = 7.79, $df = 1$, $P = 0.005$; Fig. 2c).

Biogeographical patterns of the genus as a whole

The number of *Physalis* species at a site ranged from zero to seven species. Taken as a whole, more than half of the species (15 species) occurred in Southern Mexican dry forest. The ranking of species richness of the other ecoregions was as follows: Trans-Mexican Volcanic Belt Conifer-Oak forest (13 species), Central Mexican Matorral (ten species), Sierra Madre Occidental Conifer-Oak forest (seven species), South Pacific Coast Mangrove and Dune (four species). On a per site basis, the average species richness of the sites did not differ by ecoregion (Kruskal-Wallis H test: Chi-square = 9.26, $df = 6$, $P = 0.16$). Species richness was positively correlated to elevation ($r = 0.40$, $P < 0.0001$).

The endemism scores of the sites ranged from 3.0 for sites with the most common species to 118 for the sites with the most rare species. Endemism differed by ecoregion (Kruskal-Wallis H test: Chi-square = 31.0, $df=6$, $P < 0.0001$). Post-hoc comparison of the mean ranks of endemism indicated that endemism was highest at sites in the Central Mexican Matorral and lowest at sites in Sierra Madre Conifer-Oak forest. The species richness and the endemism score of the sites was not correlated ($P = 0.22$). Endemism was not correlated with elevation ($P > 0.01$). The higher peaks of the Trans-Mexican Volcanic Belt are considered to be ecological islands. When only this ecoregion was considered, the relationship between endemism and elevation was stronger ($r = 0.60$, $P = 0.002$).

Biogeography of individual *Physalis* species

We encountered a total of 26 different *Physalis* species growing at 102 locations, and we did not encounter *Physalis* at the other 16 field sites (Table 3). This represents two-thirds of the species that are known to occur in the sampled region (Vargas *et al.* 1998, Vargas *et al.* 2003). With respect to phylogeny, these 26 species represent a broad sample of the morphologically typical *Physalis*, from more basal species like *P. acutifolia* to more derived species like *P. cinerascens* (Whitson and Manos 2005). Eleven of the 26 species have yet to be included in molecular studies of *Physalis* phylogeny. Fifteen of the 26 species were annual, and 11 of the species were perennial.

The geographic ranges of these 26 species include narrow endemics that are restricted to the state of Jalisco (two species) as well as species that are restricted to the whole of Mexico (nine species), Mexico and Central America (seven species), the US and Mexico (four species), and cosmopolitan species that occur throughout the Americas and have been introduced into the Old World (four species) (Nee 1986, Martínez 1993, Vargas 1998, Vargas *et al.* 1998, Estrada and Martínez 1999, Vargas *et al.* 2003). Most of the 13 species that are known to occur in West-Central Mexico but were not encountered in this study have limited geographic distributions (six are endemic to Jalisco or Michoacán, five occur only in Central Mexico, one extends from Mexico to Panama, and one is cosmopolitan; Vargas 1998, Vargas *et al.* 1998, Vargas *et al.* 2003).

The frequency of presence at the 118 field sites differed by *Physalis* species ($df = 13$, Chi-square = 135.85, $P < 0.0001$; Figure 3a). The percentage of the 118 study sites occupied by each species ranged from 0.85% (=1/118) for the seven most rare species to 32.20% for *P. philadelphica*. Of the 38 sites where *P. philadelphica* was present, 16 were commercial tomatillo farms, seven were feral populations of domesticated plants, and 15 were semi-domesticated plants in traditional corn fields known as “milpa”). The next most common species were *P. nicandroides* (29 sites) and *P. ampla* (30 sites). Over half of the species (17 species) occurred at fewer than ten locations, and seven species were present at only one site.

When analyzed as a group, annual species occurred at a significantly higher percentage of sites than perennial species (arcsine-square root transformed data: $t=3.94$, $P = 0.0006$). The frequency of species' occurrence differed significantly by geographic range size (Figure 3b; Kruskal-Wallis H test: Chi-square = 12.24, $df=4$, $P = 0.02$). Within West-Central Mexico, cosmopolitan species occurred at the highest percentage of sites, and species that were restricted to Jalisco, Mexico, or the US and Mexico occurred less frequently. Two of the seven species that occurred at only one of the study sites do not occur outside of the states of Jalisco. While it is

logically expected that endemic plants would not occur at most of the study sites, it is interesting that species that were restricted to US and Mexico occurred less frequently than cosmopolitan species.

The species differed with respect to realized niche breadth (Table 3). As expected, the arcsine-square root transformation of the percentage of study sites inhabited by a species was positively correlated with niche breadth (number of ecoregions: $r = 0.87$, $P < 0.0001$; arcsine-square root transformation of altitudinal gradient over which a species occurred: $r = 0.88$, $P < 0.0001$; Fig. 3c & 3d). The size of the species' geographic range was related to niche breadth (Kruskal-Wallis H test on the mean number of ecoregions: Chi-square = 13.3, $df=4$, $P = 0.01$; Kruskal-Wallis H test on mean altitudinal gradient: Chi-square = 11.43, $df=4$, $P = 0.02$; Fig. 3e & 3f). The most widely distributed species also tended to occur across the broadest assortment of habitats.

The 12 species for which we measured plant density included the nine most common species at the 118 field sites as well as three species that occurred at only one or two sites (Tables 2 and 3). There were 1 to 4 species present at the eleven locations where we measured *Physalis* plant density. There was considerable intra-specific variation in plant density at the sites. The plant density of a single species at a site ranged from 0.03 plants per m^2 to 1.83 plants per m^2 . When tested over all sites, plant density did not differ by species (Kruskal-Wallis H test: $df=11$, Chi-square = 12.8, $P = 0.30$). There was no relationship between the percentage of study sites occupied by a species and the average population density per site ($r_s = -0.05$, $P = 0.87$). There were dense patches of plants of both relatively common species (e.g. *P. lagascae*, present at 17 sites) and relatively rare species (e.g. *P. orizabe*, present at 2 sites). Plant density within a site did not differ by either lifespan (Mann-Whitney U test: mean rank of the density of annual species=6.5 versus mean rank of perennials=6.5; $U=13$; $P = 1$) or by geographic range (Kruskal-Wallis H test: Chi-square = 6.27, $df=3$, $P = 0.10$). Population density at all 11 sites was not correlated with either the number of ecoregions or the altitudinal gradient across which a species occurred ($P \gg 0.30$).

Co-occurring species

Hierarchical clustering of the *Physalis* species using the Jaccard similarity indices identified four clusters of co-occurring *Physalis* species (Fig. 4). Each cluster included both closely related and distantly related species: cluster 1a includes the sister species *P. angulata* and *P. pubescens* and cluster 3 includes the relatively basal *P. solanaceous* and the more derived *P. cinerascens* (Whitson and Manos 2005). In general, the members of each of four clusters of co-occurring

species shared similarities in their geographic range sizes. The species in cluster 1a had relatively large geographic ranges, occurred frequently at the 118 field sites, and were habitat generalists (4 ± 0.31 SE ecoregions across an average altitudinal gradient of $1,735.6 \pm 153.34$ SE m). The species in the other clusters tended to be more specialized. The species in cluster 1b had smaller geographic ranges (two species restricted to Mesoamerica and one species restricted to Mexico), and they tended to inhabit a subset of the patches where species in cluster 1a occurred (Southern Mexican Dry Forest and the Trans-Mexican Volcanic Belt Conifer-Oak Forest at an average of $1,643.2 \pm 26.27$ m in elevation). Species in cluster 2 occur in Mesoamerica (*P. leptophylla* and *P. minuta*) and throughout the Americas (*P. cordata*). They were associated with Southern Mexican Dry Forest and South Pacific Coast Mangrove, and they tended to occur close to sea level (198.78 ± 167.54 m). Species in cluster 3 occur in Mexico (*P. hastatula* and *P. patula*) or the US and Central Mexico (*P. cinerascens* and *P. solanaceous*). Within the study area, they were present at sites in the Central Mexican Matorral at $2,054.67 \pm 59.82$ m in elevation. The species in cluster 4 are Mexican endemics restricted to Trans-Mexican Volcanic Belt conifer-oak forest at $2,186.25 \pm 210.75$ m in elevation. Of those species that did not cluster with other species, six were perennials and one species was annual (*P. acutifolia*), and the species occurred at relatively few sites ($1.85\% \pm 0.51\%$ of study sites).

On commercial tomatillo farms, plants of four *Physalis* species occurred as weeds (all in cluster 1a: *P. ampla*, *P. angulata*, *P. nicandroides*, and *P. pubescens*). Six species from cluster 1a (*P. ampla*, *P. angulata*, *P. lagascae*, *P. nicandroides*, *P. pruinosa*, and *P. pubescens*), two species from cluster 1b (*P. microcarpa*, and *P. angustifolia*), one species from cluster 2 (*P. cordata*), and one unaffiliated species (*P. subrepens*) co-occurred with wild tomatillos in traditional cornfields known as “milpa”.

DISCUSSION

On a macro-scale, biogeographic patterns observed for the *Physalis* match patterns previously identified for the whole of Mexico’s flora. On average, *Physalis* endemism was highest in Central Mexican Matorral. Likewise, this ecoregion is one of the centers of endemism for the aggregate of Mexican flora. This scrubland occupies the southern most part of the Central Mexican Plateau. Geologically, it is an extension of the North American Plate. It is isolated from other xeric environments by the mountains of the eastern and western Sierra Madres and the Trans-Mexican Volcanic Belt. As a consequence of this geographic isolation, a high number of endemic taxa arose in this ecoregion, including unique genera (González and Chiang 1988, Pargas 1996). The Central Mexican Matorral is a comparatively large ecoregion of which only a

small portion was examined in this study. Sampling of a wider swath of this ecoregion could indicate that the species in cluster 3 are common throughout this environment.

The mountains in the Trans-Mexican Volcanic Belt are much younger than those of the Sierra Madre Occidental (between 1.6 to 10 million years old versus 40-140 million years old; Ferrari and López 1999). Volcanic activity in the Trans-Mexican Volcanic Belt region led to the formation of microclimates and the radiation of new species (Turner and Nesom 1993, Aljos 1996, Challenger 1998). We found that for *Physalis* the sites in the Trans-Mexican Volcanic Belt did not, on average, have a significantly higher level of endemism, but endemism within this ecoregion did increase with elevation. Likewise, five of the West-Central Mexican species that were not documented in this study are restricted to these mountains (Vargas *et al.* 2003). Correlation between taxon age and the size of a species geographic range has been observed for other taxonomic groups (Taylor and Gotelli 1994). Because the Trans-Mexican Volcanic Belt is newer, smaller, and more heterogeneous, this area may be home to more recent species radiations with even more narrow distributions than the Central Mexican Matorral endemics. Thus, future studies on the biogeography and phylogeny of *Physalis* should also target species in this ecoregion. The earliest identifiable *Physalis* fossils have been found in Europe and date to the Miocene (Collinson *et al.* 1993). Geologic activity and historical climate change may have resulted in more recent *Physalis* radiations in Mexico. Studies on the species relationships could be used to detect phylogenetic signal in the species' present day distributions.

The species examined in this study ranged from pan-global cosmopolitans to narrow endemics. The cosmopolitan species not surprisingly tended to be more common across a broader sample of the habitats represented by the 118 sites in West-Central Mexico. Some researchers have proposed that rare species with very narrow geographic distributions are adapted to extreme environments that exclude less adapted species (Drury 1974). Widespread, common species did on occasion co-occur with the most rare species, indicating that they were not excluded from any habitat class as defined by elevation or ecoregion. The number of ecoregions in which a species occurs is a crude estimate of realized niche breadth. In terms of environment, some ecoregions are more different than others. For example, although the geological history of the Trans-Mexican Volcanic Belt Conifer-Oak forest is different from the geological history of the Sierra Madre Conifer-Oak forest, environmentally these two ecoregions are not as radically different as the humid lowlands of the South Pacific Coast Mangrove and the xeric highlands of the Central Mexican Matorral, for example. Future studies of *Physalis* biogeography should examine more site-based measures of niche breadth. Field data on a species' habitat associations is only an estimate of the species' realized niche and does not necessarily reflect the species

fundamental ecological niche (*i.e.* what temperatures, light levels, drought stress, etc. that they will tolerate; Thompson *et al.* 1999).

On a local scale, plant density at a site was not correlated with range size, the frequency with which a species was present within the study area, nor niche breadth. Unaccounted for variables may be impacting local abundance. Although *Physalis* species generally tend to occur in disturbed habitats such as agricultural fields, pastures, roadsides, and construction sites, the level of disturbance with which the species are associated may vary. While we did not quantitatively assess the level of disturbance at the field sites, the results of this study are suggestive. Perennial species occurred at significantly fewer locations than annual species. Models predict that with increasing levels of disturbance, perennials are excluded (Schipper *et al.* 2001), and studies have shown that local persistence is an important feature of the population ecology of narrow endemics (Lavergne *et al.* 2004). How *Physalis* species respond to different disturbance regimes would be a site-based measure worthy of further evaluation.

Anthropogenic disturbance and agriculture in particular may influence *Physalis* distributions (Waterfall 1958). Central Mexico was an important center in the development of agriculture (Harlan 1992), and much of the land continues to be devoted to farming (Espinosa *et al.* 2004). In contrast to more northern and southern regions of the Americas where the majority of weedy species are introduced, the community of weedy plants that co-occurs with agriculture in Mexico is predominately native (Rzedowski 1993, Vibrans 1998). Alongside the development of agriculture, some native species have adapted to occur in conjunction with anthropogenic disturbance (Vibrans 1998). Plants in the genus *Physalis* are an example of native taxa that have adapted to survive within agricultural ecosystems (Rzedowski 1993). The fruit of many *Physalis* species are edible (Martínez 1993), and archaeological evidence indicates that *Physalis* fruits have been used as food for thousands of years. The use of the tomatillo may have predisposed Mexican society to domestication of the tomato, which is an introduced plant in Mexico (Jenkins 1948). Because *Physalis* occurs in conjunction with disturbance and because it has been used as a food plant for millennia, the present-day distribution of some *Physalis* species has been highly influenced by humans (Waterfall 1967).

The differences that we found in the species' habitat associations, niche breadth, and geographic range sizes prompt the question as to what is the underlying cause. Many hypotheses have been put forward to explain differences between rare and common species (Bevill and Louda 1999, Murray *et al.* 2002). Species with large range sizes may be able to tolerate a wider variety of environmental conditions (Gaston and Spicer 2001) or they may have greater dispersal or competitive abilities (Walck 1999). The genus *Physalis* can be used as a model for future

attempts to answer these questions. Important taxonomic and systematic work on this genus continues. Phylogenetic studies will contribute to the understanding of species relationships. Studies of differences among species in life history traits (see Chapter 4) may explain some differences in distributions.

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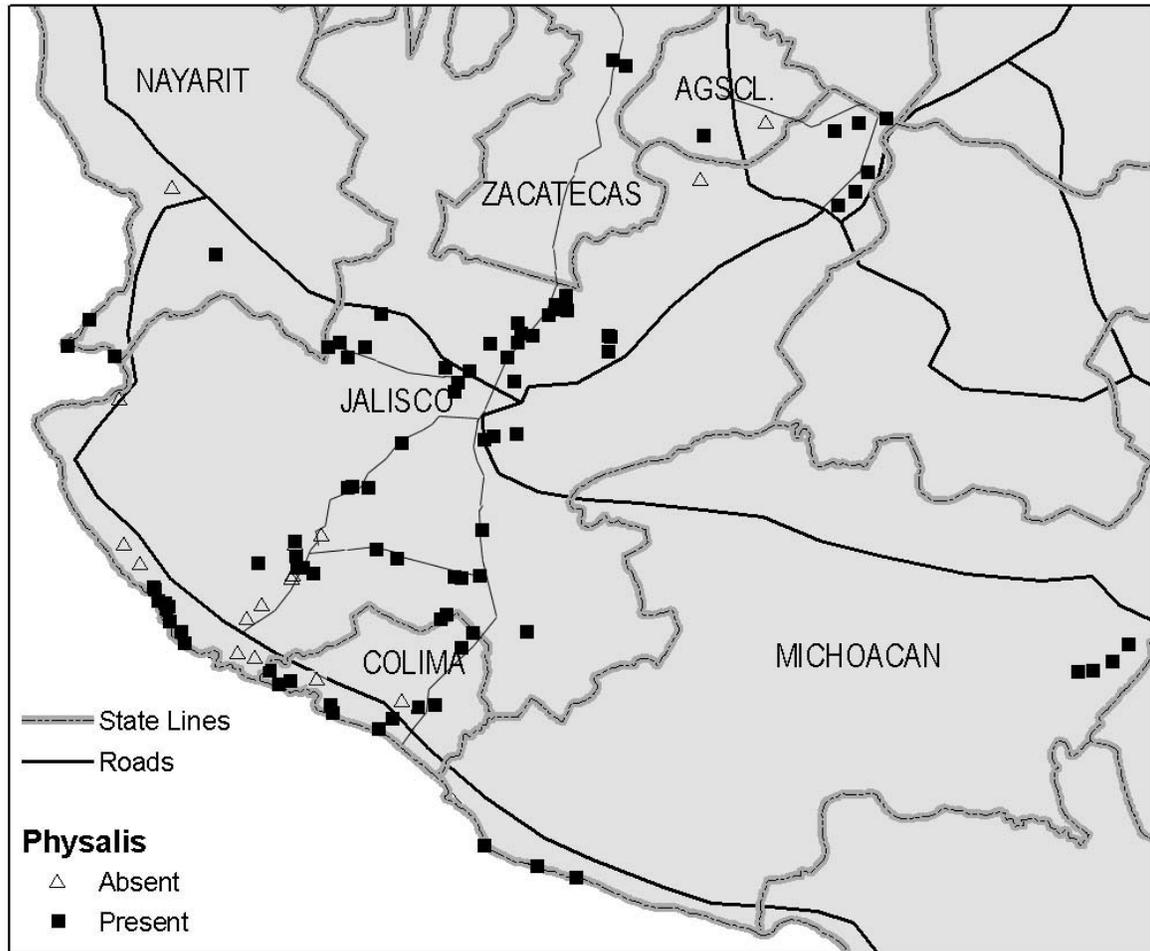


Fig. 1. Distribution of *Physalis* species at 118 field sites in West-Central Mexico. Open triangles indicate that no plants of any *Physalis* species were detected at the site. Closed squares indicate that plants of at least one *Physalis* species were present at the site.

Table 1. Overview of major natural features, ecoregions, and dominant vegetation classes in West-Central Mexico (per Rzedowski 1978, Olson and Dinerstein 1998).

Natural Features	Ecoregion	Dominant Vegetation
Coastal plain	South Pacific Coast Mangroves & Dunes (SPCM)	Mangroves & Palm
Trans-Mexican Volcanic Belt	Southern Mexican Dry Forest (SMD)	Deciduous and sub-deciduous tropical forest
"	Trans-Mexican Volcanic Belt Conifer-Oak Forest (TMVB)	Pine and oak
Sierra Madre Occidental	Southern Mexican Dry Forest (SMD)	Deciduous and sub-deciduous tropical forest
"	Sierra Madre Occidental Conifer-Oak Forest (SMO)	Pine and oak
Central Mexican Altiplano	Central Mexican Matorral (CMM)	Sub-tropical scrub, bunch grassland, and thorn forest

Table 2. Descriptions of the eleven localities at which we measured *Physalis* plant density. All sites were in Jalisco, Mexico.

Location (Municipality, Town)	Longitude	Latitude	Elevation (m)	Measurement dates	Ecoregion	Disturbance	<i>Physalis</i> species
Lagos de Moreno, Presa de Cuarenta	-101.79987	21.455239	1,931	10/16/2004	Central Mexican Matorral	Pasture	<i>P. angulata</i>
Ojuelos de Jalisco, Ojuelos de Jalisco	-101.56688	21.875993	2,173	10/16/2004	Central Mexican Matorral	Roadside	<i>P. patula</i>
Cocula, Arroyo Colorado	-103.90833	20.30175	1,706	10/9/2004	Trans-Mexican Volcanic Belt Conifer-Oak Forest	Corn field	<i>P. ampla</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i> , <i>P. pruinosa</i>
Tenamaxtlan, Colotitlan	-104.16667	20.083333	1,396	10/9/2004	Southern Mexican Dry Forest	Corn field	<i>P. angulata</i> , <i>P. lagascae</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i>
San Gabriel, Izote	-105.27056	21.630528	1,554	10/10/2004	Trans-Mexican Volcanic Belt Conifer-Oak Forest	Pasture	<i>P. orizabe</i>
Ciudad Guzmán, Puerto Floripondio	-103.62053	19.645306	2,397	10/10/2004	Trans-Mexican Volcanic Belt Conifer-Oak Forest	Logging road	<i>P. lignescens</i>
La Huerta, Xametla	-105.08508	19.53725	2.5	10/14/2004	South Pacific Coast Mangrove & Dune	Beach	<i>P. minuta</i>
La Huerta, Chamela	-105.04925	19.524194	35	10/14/2004	Southern Mexican Dry Forest	Riverbed	<i>P. cordata</i>
La Huerta, Estación de Biología Chamela	-105.03694	19.510861	70	10/6/2004	Southern Mexican Dry Forest	Forest	<i>P. leptophylla</i>
La Huerta, Careyitos	-105.02606	19.437639	10	10/13/2004	South Pacific Coast Mangrove & Dune	Beach	<i>P. minuta</i>
Mazanillo, near Puente Tepelcates	-104.25265	19.03315	13	10/15/2004	South Pacific Coast Mangrove & Dune	Corn field	<i>P. lagascae</i>

Table 3. The 26 *Physalis* species that occurred at the 118 field sites in West-Central Mexico and aspects of their biogeographies.

<i>Physalis</i> species	Habit	Geographic range ¹	Ecoregion ²	Altitudinal gradient (max-min)	Percentage of sites occupied	Density (plants per m ²)
<i>P. acutifolia</i>	Annual	Southern US through central Mexico	SPCM	10 m to 54 m	2.54	.
<i>P. ampla</i>	Annual	Mexico	TMVB, SMO, SMD	690 m to 1,934 m	25.42	0.09
<i>P. angulata</i>	Annual	North and South America; introduced Old World Tropics	CMM, SMD, TMVB, SMO	890 m to 2,415 m	18.64	0.12
<i>P. angustiphysa</i>	Perennial	Mesoamerica	TMVB, SMD	1,600 m to 1,706 m	1.69	.
<i>P. chenopdifolia</i>	Perennial	Mexico	SMD	.	0.85	.
<i>P. cinerascens</i>	Perennial	US and Mexico	CMM	.	0.85	.
<i>P. cordata</i>	Annual	North and South America; introduced Old World Tropics	TMVB, SMD, SPCM	2 m to 1,850 m	15.25	0.03
<i>P. glutinosa</i>	Perennial	Mexico	CMM	.	0.85	.
<i>P. hastatula</i>	Perennial	Mexico	CMM	1,931 m to 2,216 m	2.54	.
<i>P. hederifolia</i>	Perennial	American southeast and Mexico	SMD	900 m to 1,200 m	2.54	.
<i>P. lasgascas</i>	Annual	Mesoamerica; likely introduced to Old World	CMM, SMD, SPCM, TMVB, SMO	13 m to 1,934 m	14.41	0.62
<i>P. leptophylla</i>	Annual	Mexico	SMD	35 m to 70 m	2.54	0.43
<i>P. lignescens</i>	Perennial	Jalisco endemic	TMVB	.	0.85	0.06
<i>P. melanocystis</i>	Perennial	Mesoamerica	SMD	.	2.54	.
<i>P. microcarpa</i>	Annual	Mesoamerica	TMVB, SMD	1,292 m to 1,806 m	2.54	.
<i>P. minuta</i>	Annual	Mesoamerica	SMD	2 m to 29 m	7.63	0.53
<i>P. nicandroides</i>	Annual	Mesoamerica	CMM, SMD, TMVB, SMO	890 m to 2,415 m	24.58	0.98
<i>P. orizabe</i>	Perennial	Mexico	TMVB	1554 m to 2,397 m	1.69	0.82
<i>P. patula</i>	Annual	Mexico	CMM	2,173 m to 2,216 m	1.69	0.31
<i>P. philadelphica</i>	Annual	Mexico, introduced US and Central America	CMM, SMD, SMO, TMVB	10 m to 2,415 m	32.20	0.32
<i>P. pruinosa</i>	Annual	North and South America	SMD, TMVB, SMO	35 m to 1,850 m	13.56	0.41
<i>P. pubescens</i>	Annual	North and South America; introduced Old World Tropics	CMM, TMVB, SMO, SMD	35 m to 1,960 m	17.80	.
<i>P. solanaceous</i>	Annual	Southern US and Mexico	CMM	1,000 m to 2,415 m	3.39	.
<i>P. subrepens</i>	Perennial	Mexico	TMVB	.	0.85	.
<i>P. sulphurea</i>	Annual	Mexico	TMVB, SMD	.	0.85	.
<i>P. waterfalli</i>	Perennial	Jalisco endemic	SMD	.	0.85	.

¹ Waterfall 1967, Nee 1986, Martínez 1993, Martínez 1998, Vargas 1998, Vargas *et al.* 1998, Vargas *et al.* 2003. ² Central Mexican Matorral (CMM), Southern Mexican Dry Forests (SMD), Sierra Madre Occidental (SMO), Southern Pacific Coast Mangrove and Dunes (SPCM), Trans-Mexican Volcanic Belt conifer-oak forest (TMVB).

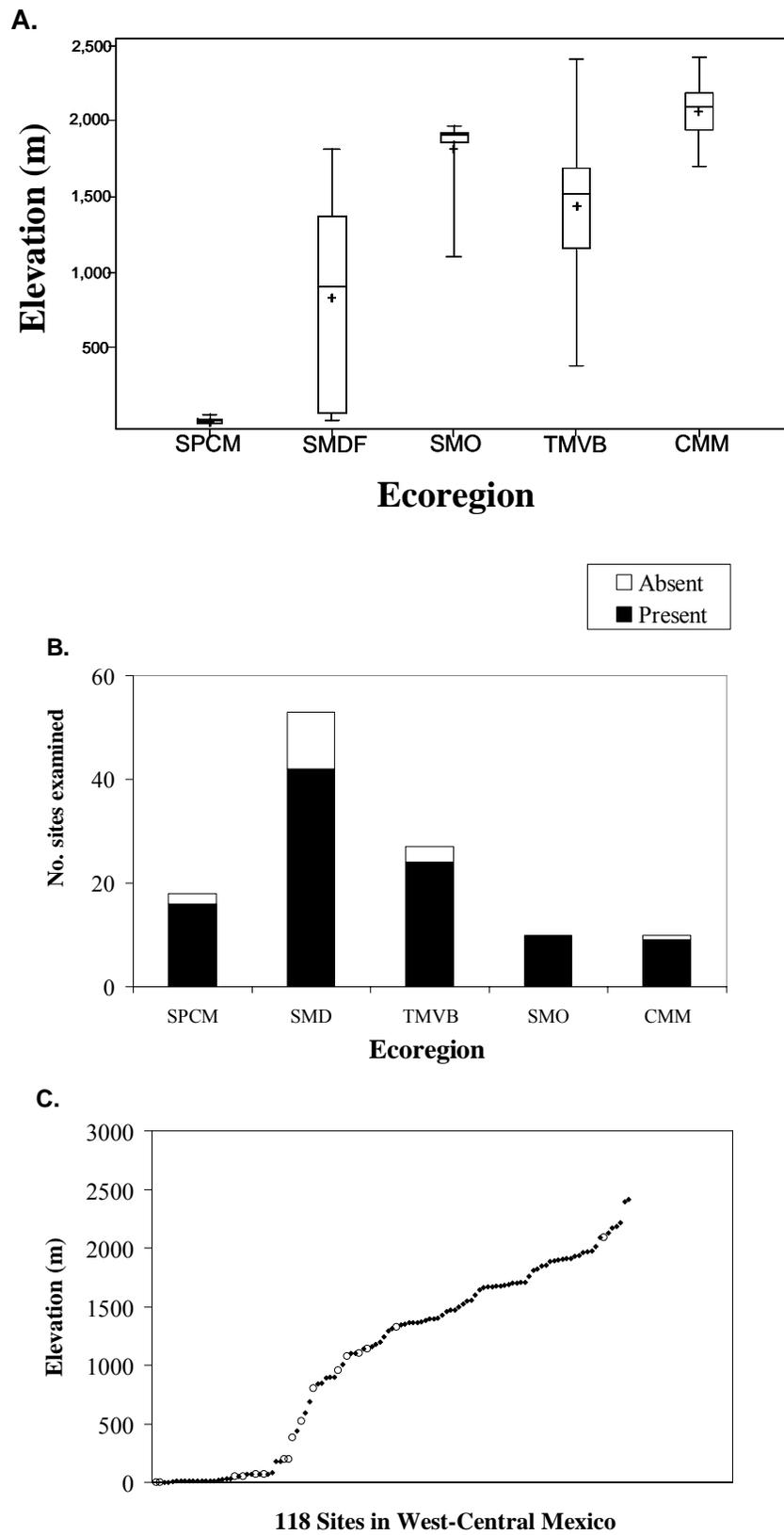


Fig. 2. A. The elevational gradient across which each ecoregion occurred. *Physalis* distribution by B. ecoregions and C. elevations.

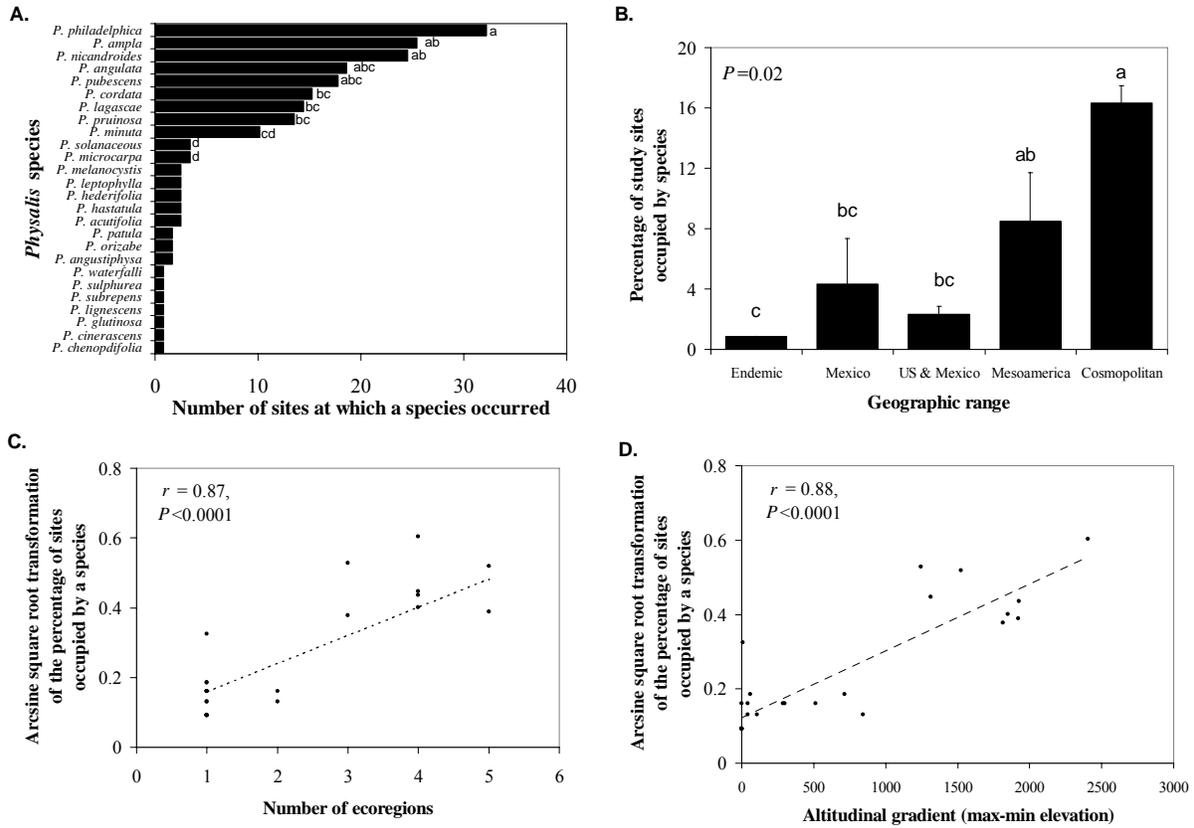


Fig. 3. Biogeography of individual *Physalis* species. A. The species occupied a significantly different percentage of the 118 study sites. B. The percentage of field sites at which a species occurred differed significantly by the size of the species' geographic range size. The percentage of study sites occupied by a species was positively correlated to both C. the number of ecoregions in which a species occurs and D. the altitudinal gradient across which a species occurred.

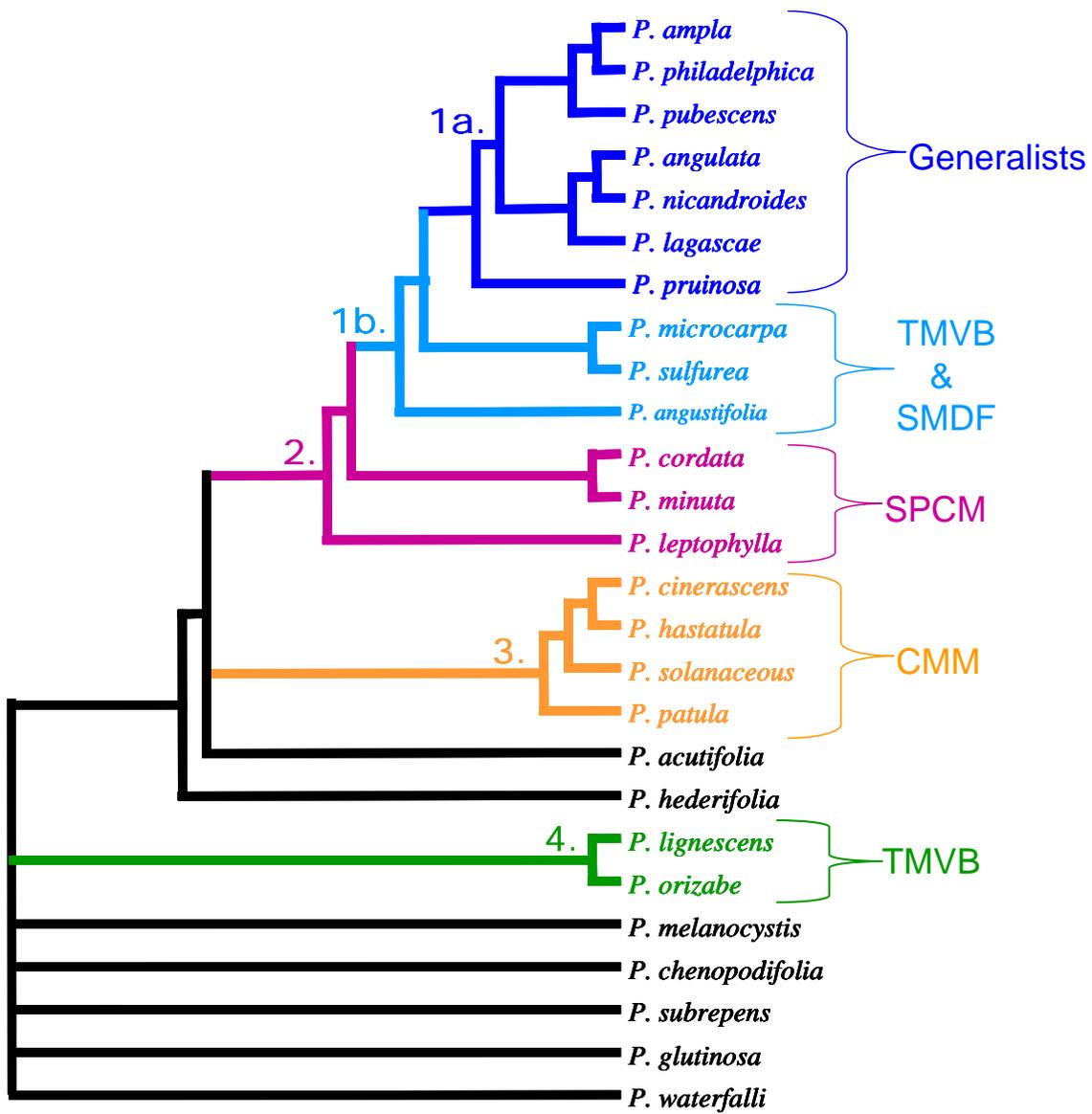


Fig. 4. Dendrogram of co-occurring *Physalis* species based on Jacard similarity indices. Colors and numbers indicate groups of species with similar geographic distributions. Central Mexican Matorral (CMM), Southern Mexican Dry Forests (SMD), Sierra Madre Occidental (SMO), Southern Pacific Coast Mangrove and Dunes (SPCM), Trans-Mexican Volcanic Belt conifer-oak forest (TMVB).

4. VARIATION AMONG ELEVEN SPECIES OF
PHYSALIS L. (SOLANACEAE) IN LIFE HISTORY

ABSTRACT

Aim A plant species' life history characteristics may help to determine how common and widespread it is. Species in the *Physalis* L. (Solanaceae) genus differ widely with respect to their biogeographies. The objective of this study was to characterize the life histories of eleven contrasting *Physalis* species, identify trade-offs among life history characteristics, and assess whether some life history traits are correlated to the species' geographic distributions.

Location Between 18° to 23° North and 100° to 105° West in the Mexican states of Aguascalientes, Colima, Guerrero, Jalisco, Michoacán, Nayarit, and Zacatecas.

Methods Over the course of three separate years, we took data on seasonal changes in fruit production over time of wild *Physalis* growing at 18 sites in West-Central Mexico. Likewise, we took data on changes in fruit production over time and total lifetime fruit production of plants growing in two common gardens, one at 1,500 m and another at 60 m in elevation. Additionally, we took detailed measurements of biomass allocation and the fruit characteristics of the common garden and greenhouse plants. Using data collected on plants in one of the common garden studies, we characterized life history strategies using principal components analysis on the measurements of fruit production, biomass allocation, and fruit characteristics. We used the species' factor scores for the principal components in statistical tests to assess the relationship between life history and biogeography.

Results

For *Physalis* species that occurred at both lowland and highland field sites, fruit production differed by elevation. The number of fruits per senescent of a species growing in the field was not correlated with lifetime fruit production by plants growing in the common gardens. The fruit diameter of plants in the two common gardens and the greenhouse were positively correlated.

We detected trade-offs between fruit characteristics (many small fruit with few seeds versus fewer, larger fruit with many seeds) and between allocation to growth versus reproduction. Species that tend to produce fewer, larger fruit with many seeds tended to have larger geographic ranges and are more common in West-Central Mexico across a wide elevation gradient. The growth versus reproduction trade-off was not associated

with the species' biogeography. While a set of eight and a set of two of the study species often co-occur in the field, none of the principal components identified in this study were significant predictors of species co-occurrence.

Main Conclusions This study indicates that seed packaging is associated with *Physalis* species' geographic distributions on both a local scale within West-Central Mexico and on a global scale. Within a species, the consistency of fruit size across rearing environments contrasts with the variation that we observed in fruit production across environments. This suggests that fruit size may be an important factor governing the dispersal of *Physalis* seeds, either through attraction of seed dispersers or through protection of the seeds from environmental hazards.

Key words: Fruit production, seeds, biomass allocation, *Physalis*, Mexico, geographic range

INTRODUCTION

At its most basic, a plant's life history involves growth, reproduction, and senescence. Each of these components is multi-faceted (Enright *et al.* 1995, Franco and Silvertown 1996). For example, plant reproduction is a function of several interdependent factors: the number and weight of seeds per fruit, the number of fruits per infructescence, and the number of infructescences per plant (Venable 1996, Jakobsson and Eriksson 2000). Life history components often co-vary and trade-offs can exist among life history elements (MacArthur 1962). For example, there is typically a negative correlation between seed size and both the number of seeds produced (Jakobsson and Eriksson 2000) and the average dispersal distance of seeds (Venable and Brown 1988). Specific life history traits often reflect short- and long-term selection pressures from abiotic factors (*e.g.* shade, temperature, salinity, drought tolerance) and biotic associations (*e.g.* pollinators, seed dispersers, competitors, herbivores, pathogens) that a plant population experiences. For example, at the recruitment stage, species with larger seeds tend to be more able to withstand drought, shade, mineral nutrient deficiency, competition with other plants, and folivory (Westoby *et al.* 1996).

The geographic distribution of individual species varies at many spatial scales (*e.g.* the size of a species' geographic range, the frequency with which a species occurs at sites within its range, and the density of individuals of the species at a particular site). The frequency with which a species occurs at sites within its geographic range tends to be positively correlated with the overall size of the species' geographic range (Brown 1984). Some studies have shown

correlations between species' life history characteristics and their geographic distributions (Bevill and Louda 1999, Murray *et al.* 2002).

Both the life history and the geographic distribution of a species may be related to its phylogeny (Silvertown and Dodd, 1996). For example, the size of a species' geographic range can be correlated to taxon age (Taylor and Gotelli 1994). Thus, care must be taken to determine whether shared life history traits and/or overlapping geographic distributions are due to common ancestry or convergent evolution (e.g. Brändle *et al.* 2003, Lavergne *et al.* 2003, Aguinagalde 2005). If a phylogenetic tree is unavailable, one conservative way to conduct comparative studies of life history traits is to compare closely related taxa such as congeners (Silvertown and Dodd 1996).

Plant species in the *Physalis* L. (Solanaceae) genus have contrasting biogeographies. The sizes of the geographic ranges of individual *Physalis* species vary by several orders of magnitude (Waterfall 1958, Waterfall 1967, Nee 1986, Martínez 1993, Vargas *et al.* 1998, Estrada and Martínez 1999, Vargas *et al.* 2003). Seventy of the approximately 90 (estimates range from 75 to 140) species in the genus are endemic to Mexico (Waterfall 1967, Vargas *et al.* 2003, Whitson and Manos 2005). In Mexico, members of the *Physalis* genus occur in all but the most extreme environments, but the *Physalis* species composition at a site is affected by habitat characteristics (Vargas *et al.* 2003). The frequency with which individual *Physalis* species occur in Mexico, and the realized niche breadth of the individual species varies (Vargas *et al.* 2003; refer Chapter 3). The most common and geographically widespread *Physalis* species tend to occur across the widest range of habitats but this is not always the case (refer Chapter 3). The relationship between *Physalis* species life history characteristics and their geographic distributions has not been examined.

The purpose of this study was to characterize the life histories of eleven annual *Physalis* species, identify trade-offs among life history characteristics, and assess whether some life history traits are correlated with the species' biogeographies. This aim was achieved through a combination of observations in the field, two common gardens, and the greenhouse. To gauge intrinsic differences in the species' life histories, we followed fruit production by wild *Physalis* plants from recruitment to senescence at 18 field sites and in two common gardens in West-Central Mexico.

METHODS

Study plants

Species descriptions. We examined the life histories of eleven annual *Physalis* species: *P. ampla*, *P. angulata*, *P. cordata*, *P. lagascae*, *P. microcarpa*, *P. minuta*, *P. nicandroides*, *P. philadelphica* (the tomatillo), *P. pruinosa*, *P. pubescens*, and *P. solanaceous* (Table 1). The nine study species that have been included in phylogenetic studies are spread evenly across the set of annual species that forms the base of the *Physalis* phylogenetic tree, with *P. solanaceous* as the most basal species and *P. nicandroides* as the most derived (Martínez 1993, Whitson and Manos 2005).

Six of the study species occur throughout the Americas and have been secondarily introduced into the old world, and the other five species have much smaller geographic ranges (Table 1; Martínez 1993, Vargas *et al* 1998, Vargas 2003). In a survey of field populations of 26 *Physalis* species at 118 field sites in West-Central Mexico, nine of the species (*P. ampla*, *P. angulata*, *P. cordata*, *P. lagascae*, *P. minuta*, *P. nicandroides*, *P. philadelphica*, *P. pruinosa*, and *P. pubescens*) were relatively common, occurring at 12 to 38 of the field sites (Chapter 3). In contrast, plants of *P. microcarpa* and *P. solanaceous* occurred at three and four sites respectively. Eight of the study species are habitat generalists, occurring across an average altitudinal gradient of $1,735.6 \pm 153.34$ SE m (Vargas *et al.* 2003; see also Chapter 3). The habitat associations of *P. microcarpa*, *P. minuta*, *P. solanaceous* are not as generalized: *P. minuta* plants are common only on beaches, sand dunes, and rocky outcrops in lowland coastal locations; *P. microcarpa* plants occur in Southern Mexican Dry Forest and the Trans-Mexican Volcanic Belt at an average of $1,643.2 \pm 26.27$ m in elevation; and *P. solanaceous* plants occur only in the Central Mexican Matorral at $2,054.67 \pm 59.82$ m in elevation. Out of the 11 species examined in this study, a set of seven species (*P. ampla*, *P. angulata*, *P. lagascae*, *P. nicandroides*, *P. philadelphica*, *P. pruinosa*, and *P. pubescens*) and another set of two species (Cluster 2: *P. cordata* and *P. minuta*) frequently co-occur at the same sites in the field.

Field

Study area and field sites. Over the course of three years, we followed field populations of ten of the eleven study species at 18 locations between $18^\circ - 21^\circ$ North and $100^\circ - 105^\circ$ West in the West-Central Mexican states of Colima and Jalisco (Fig. 1, Table 2). This geographic area includes the narrow band of the Pacific Coastal lowlands, the Occidental Sierra Madre, the Trans-Mexican Volcanic Belt, and the Central Mexican Plateau (Rzedowski and McVaugh 1966, Olson and Dinerstein 1998, Olson *et al.* 2001). The field sites that we selected were accessible locations with moderate levels of disturbance typical of the habitats in which *Physalis* occurs (*e.g.*

roadsides, trails, pasture, stream banks, coastal dunes, cornfields, and tomatillo fields; Waterfall 1958, Vargas *et al.* 2003). The study sites represented a broad spectrum of the habitats with which the eleven species are associated in West-Central Mexico. They were located between sea level and 2,000 m in elevation (Table 2). Multiple *Physalis* species were present at all but four of the 15 locations. Because of the considerable distance between some of the field sites (up to 1,500 km), we took data on plants at most of the 15 field sites in a subset of the three years (Table 2).

Data collection on field plants. During each round of observations at a field site, we searched for wild *Physalis* over a minimum area of 200 m², and we noted which species were present. At each site, we walked in a random direction, and we took data approximately every 5 m on a randomly selected plant, without reference to plant size. We tried to collect data on a minimum of 24 plants per date per locale and multiple individuals of each species per site, but this was not always possible. For each individual that we examined, we counted the total number of fruits attached to the plant. Since mature or damaged fruit often abscise (Sisterson and Gould 1999, Petzold *et al. in preparation*) and remain close to the plant canopy, we also counted the abscised fruits associated with each plant.

Study period. In Mexico, seasonal change in the abundance of herbaceous plants like *Physalis* tends to follow variation in rainfall rather than temperature. The rainy season runs from May to October (Bobbink and Heil 2003). In preliminary scouting trips from June to mid-July, we did not encounter *Physalis* plants. Starting in the first week of August, we repeatedly examined the plants at the field sites on a rotating basis until the onset of senescence. As of the latter half of October, the populations of wild plants were in decline, and plants at most sites had senesced. We evaluated fruit production of wild *Physalis* plants over a 108 day interval, from August 6th to November 22nd.

Common gardens and greenhouse studies

Sites. To examine the expression of the species' life histories in shared environments, we conducted common garden studies at two different sites in West-Central Mexico and we also reared plants in a greenhouse setting. In 2003, plants of eight species (*P. ampla*, *P. angulata*, *P. lagascae*, *P. minuta*, *P. nicandroides*, *P. philadelphica*, *P. pruinosa*, and *P. pubescens*) were grown in a common garden at the Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA) of the Universidad de Guadalajara, Zapopán, Jalisco, Mexico (20° 47' 0" N, 103° 30'

0" W). This site is located at 1,550 m in elevation in Southern Mexican dry forest, and it has a semi-arid tropical climate (Tereshchenko and Filonov 2001). Annually, this area receives a little over 900 mm in precipitation, principally between June and October. Natural populations of feral tomatillos, *P. ampla*, *P. angulata*, *P. lagascae*, *P. microcarpa*, *P. nicandroides*, and *P. pubescens* occur within 30 km of CUCBA (Vargas *et al.* 2003). Plants of *P. minuta* and *P. solanaceous* were not locally present.

In 2004, plants of ten species (*P. ampla*, *P. angulata*, *P. cordata*, *P. lagascae*, *P. microcarpa*, *P. minuta*, *P. nicandroides*, *P. philadelphica*, *P. pruinosa*, and *P. solanaceous*; *P. pubescens* was not grown at this location) were grown in a common garden at the Estación de Biología Chamela (EBC), a research facility affiliated with the Universidad Nacional Autónoma de México. This research station is located in the lowlands of the Pacific Coast of Jalisco, México (70 m in elevation, 19°30'N, 105°03'W). The climate at the research station is tropical, warm subhumid with a pronounced dry season (Lott 1993). The dominant vegetation is tropical deciduous forest with much of the flora dormant during the dry season (Lott 1987). Populations of *P. cordata*, *P. lagascae*, *P. minuta*, the cultivated tomatillo, *P. pruinosa* and *P. pubescens* are present in the immediate vicinity of the EBC (Vargas Ponce *et al.* 2003).

In 2005, 10 individuals of ten of the study species (*P. ampla*, *P. angulata*, *P. cordata*, *P. microcarpa*, *P. minuta*, *P. nicandroides*, *P. philadelphica*, *P. pruinosa*, *P. pubescens* and *P. solanaceous*) were reared in a greenhouse at the Max Planck Institute for Chemical Ecology (MPICE), Jena, Germany.

Plant rearing and experimental designs. Germplasm for the plants used in the common gardens and the greenhouse study came from seeds and seedlings of wild and domesticated plants collected from the field in West-Central Mexico (Appendix A). Seedlings were initially kept in 10 cm deep trays. Once the seedlings had four or more true leaves, the plants were transferred to 3.75 L containers. Thereafter, the plants received liquid fertilizer on a weekly basis (1 tbsp of 15-30-15 NPK per gallon of water). At CUCBA and EBC, the potted plants were kept outside where they experienced natural sunlight and rainfall. They were also watered as needed. Insect pollinators and herbivores from the surrounding environment occasionally interacted with the common garden plants (Appendix A). At MPICE, the plants were grown in a greenhouse at 22±1°C, 55±5 Rh, and 14:10 light dark cycle. Some of the plants at MPICE were infected with a pathogen. Plants showing symptoms of pathogen infection were discarded and replaced with healthy plants.

At CUCBA, the plants remained in pots until they were approximately 50 days old, the age at which individuals of most species become reproductive (Cartujano *et al.* 1985; see also Chapter 4). The plants had not become pot bound. The plants were then transplanted into the field site in eight randomized blocks with nine plants of each species per block and 0.80 meter spacing between plants. Two thirds of the plants of each species in each block experienced herbivore exclusion treatments, but these plants are not dealt with here. Only the 168 untreated control plants are addressed in the current study. Plants that died within the first ten days of the study were replaced. For the first two weeks following transplantation, weeds were removed from an area with a 50 cm radius around each of the experimental plants. The CUCBA study took place over a 74 day period between September 1st and November 14th of 2003.

The EBC common garden study ran from August 5th to November 16th of 2004. For the full duration of the EBC study, the plants were maintained in 3.75 L containers on wooden benches set out on a lawn area at the research station. The plants were arranged in five randomized blocks with two plants of each species per block. One plant in each pair experienced a simulated herbivory treatment, but only the untreated control plants are addressed in the current study. When the common garden studies ended in November, the rains at each location had stopped and most of the common garden plants had either died or were showing symptoms of senescence. At this time, the wild *Physalis* plants that had been growing in the vicinity of the common gardens had senesced.

At MPICE, seeds for the experiments were sown in four blocks (January 13th, February 18th, February 22nd, and March 9th of 2005). Once each study plant had a minimum of four flowers (37 - 71 after seeds were sown, depending on the species), we marked four to 15 flowers with no signs of previous pollination (open flowers with erect petals, no pollen grains on the stigma, and no withering of the flower). Since some of the species are self-incompatible (Menzel 1951, Pandey 1957, Whitson 2005), we cross-pollinated flowers with pollen from a randomly selected conspecific plant (per Whitson 2001). Following the first round of hand pollination of each plant, we marked four to 15 additional flowers every four days for a period of 12 days. Due to abscission of some flowers and fruits, not all marked inflorescences produced fruit. All of the marked fruits on an individual plant were harvested at the same time. Harvests of the plants' fruits were staggered such that the fruits collected represented the whole process of fruit maturation for each species (ranging from 4 day old fruit to 31 day old fruit). Twenty days after pollination, the fruits had attained full size (Chapter 4). Only fruits that were 20 days old or older were used in the analyses.

Data collection on common garden and greenhouse plants. We sought to characterize fruiting by the CUCBA and EBC common garden plants from germination to senescence. To examine changes in fruit production over time, we counted the attached fruits and abscised fruits associated with each plant on a weekly basis.

After collecting fruiting data on the EBC plants for the last time on November 16th of 2004, we harvested the roots, stems, leaves, and fruits of each plant. We then dried ($42.15 \pm 0.03^\circ\text{C}$ and $29.76 \pm 0.03\%$ relative humidity) and weighed the plant material. To estimate the biomass of abscised fruits, we multiplied the total number of abscised fruit by each plant by the average dry weight of a fruit on that plant. The total mass of a plant was the sum of the masses of its roots, stems, leaves, and fruits, including the estimated mass of abscised fruits of that plant. We calculated the percent of the total mass that was accounted for by the roots, stems, leaves, and fruits.

To assess the contribution of a single fruit to the overall productivity of the plants of each species, we measured characteristics of the fruits and the seeds. We measured the diameter of mature, full-sized fruits from the floral abscission scar to the point of attachment to the peduncle. In the common gardens and the greenhouse, the method of fruit selection differed somewhat. For CUCBA plants, we measured 20 undamaged abscised fruit. *Physalis* plants typically only abscise fruit without damage when they are ripe or overripe. FOR EBC plants, we measured 20 fruits of senescent plants. For MPICE plants, we measured fruits that had ceased to expand in size (fruits that were 20 days old or older). For the EBC plants, we counted the number of seeds per fruit for mature fruit of each species. To estimate the total number of seeds produced per plant over the course of a season, we multiplied each individual's total fruit production by the average number of seeds per fruit for the species. We weighed forty randomly selected seeds of EBC plants of each species.

Statistical analyses

Life history characteristics. The mean counts of fruits of field and common garden plants were natural log-transformed to approximate normality. Using analysis of variance (SAS, proc glm), the counts of fruits associated with each plant in the field and in the common gardens was modeled using the *Physalis* species fixed effect, the continuous variable time, the quadratic function of time (due to abscission or senescence, the number of fruit might decrease), and all of their interactions. Analyses were carried out separately for plants in the field, at CUCBA, and at EBC. We evaluated the changes in the numbers of fruit per plant over time relative to the julian date of data collection for the field plants and relative to plant age for the common garden plants.

For the species that occurred from sea level to over 1,800 m in elevation (*P. cordata*, *P. lagascae*, *P. pruinosa*, and *P. pubescens*), we carried out another set of analyses in which the elevation of the sites (below 500 m or above 500 m) was included as a fixed variable in the model described above.

For EBC and CUCBA plants, we estimated the total lifetime fruit production by each of the common garden plants by summing the total number of fruits that were attached to each plant on the last date that the plant was alive to the total of the number of fruits that had been abscised by the plant over the course of the common garden study, and we used analysis of variance to test whether total fruit production per common garden plant differed by *Physalis* species.

As the rainy season ended in mid-October, plants began to senesce. Given that senescent plants were in comparable phenological states, we calculated *lsmeans* for the number of fruits per field plant. We tested for correlations between senescent field plants and total lifetime productivity of common garden plants using Spearman's Rho.

We used analysis of variance to test whether either the total mass or the percentage of the plant's total mass accounted for by roots, stems, leaves, or fruits differed by *Physalis* species.

Using analysis of variance, we carried out separate tests on plants at CUCBA, EBC, and MPICE to determine if *Physalis* species had an effect on fruit diameter. We then assessed whether or not the diameters of fruits of each species grown in the different environments were correlated using Spearman's Rho. We used general linear models to test whether the number of seeds per fruit or seed weights of EBC plants differed by species.

Trade-offs in life history characteristics. We constructed a correlation matrix for the measurements of phenology, fruit production, biomass allocation, and fruit characteristics (with adjustment for multiple comparisons using sequential Bonferroni). We identified life history strategies using principal components analysis on these variables. Principal components with eigen values greater than 1 were considered significant, and major relationships had eigen vectors of greater than 0.35.

Relationship between life history characteristics and biogeography. We used the species' factor scores of the principal components in statistical tests to assess the relationship between life history and biogeography. The Kruskal Wallis test was used to determine if the mean of factor scores of each of the principal components differed by geographic range (cosmopolitan versus narrowly distributed). Using Spearman's Rho, we tested for correlation between the principal components and the frequency with which the species occurred at sites in West-

Central Mexico. Likewise, we used Spearman's Rho to test for correlation between the factors scores and the elevation gradient across which the species occur. We tested whether the mean factor scores differed for the species in the co-occurring clusters using the Kruskal Wallis test.

All statistical analyses were conducted using SAS software (SAS 8.2 for Windows, SAS Institute, 2001). For significant effects in each of the analyses of variance, post hoc tests were performed using Tukey's *hsd*. In all tests, the significance level was set at $\alpha = 0.05$ and ± 1 standard error (SE) values are presented for estimated mean values.

RESULTS

Life history characteristics

Within the field study area, flowers and fruits were produced by at least some naturally-growing plants of *Physalis* species from early August to late November. The number of fruits per field plant differed among *Physalis* species and it was curvilinearly affected by date (Table 3a; Fig. 2). The number of fruit per field plant at senescence ranged from 3.5 ± 0.2 (*P. minuta*) to 72.70 ± 0.5 (*P. solanaceous*; Table 4).

For the four species that occurred at sites from sea level to over 1,800 m, the number of fruits per plant was significantly affected by elevation, species, and the elevation x species interaction, and it was curvilinearly affected by date (Table 5; Fig. 2). At senescence (October 16th), plants of *P. cordata* and *P. lagascae* tended to have fewer fruit at sites below 500 m than at sites above 500 m (least square means of *P. cordata*: $< 500 \text{ m} = 3.9 \pm 1.2$ vs. $> 500 \text{ m} = 11.0 \pm 1.4$ fruits per plant; *P. lagascae*: $< 500 \text{ m} = 4.6 \pm 0.2$ versus $> 500 \text{ m} = 26.1 \pm 0.2$ fruits per plant). The opposite was true for *P. pubescens* plants ($< 500 \text{ m} = 22.81 \pm 0.4$ versus $> 500 \text{ m} = 12.9 \pm 0.3$ fruits per plant). The number of fruit per *P. pruinosa* plant did not differ by elevation ($< 500 \text{ m} = 13.80 \pm 0.5$ versus $> 500 \text{ m} = 14.18 \pm 0.32$ fruits per plant).

For plants grown at CUCBA, the number of fruit associated with each plant over time differed linearly by *Physalis* species, date, and the *Physalis* species x date interaction (Table 3a; Fig. 2). Lifetime fruit production differed by species (Table 3b). Total fruit production per plant ranged from 5.0 ± 3.5 (*P. cordata*) to 156.0 ± 26.8 (*P. solanaceous*; Table 4).

The number of fruits per EBC plant differed by *Physalis* species, and it was affected curvilinearly by plant age (Table 3; Fig. 2). The sum total number of fruit produced over the course of the life of the EBC common garden plants differed by *Physalis* species ($df = 9, 36, F = 4.98, P = 0.0002$; Table 6). Total fruit production per EBC plant ranged from 6.9 ± 1.6 (*P. angulata*) to 51.9 ± 12.9 (*P. ampla*; Table 4).

There was no correlation in the mean number of fruit per senescent field plant of a species and total fruit production by plants of the species in the CUCBA common garden ($r_s = 0.11$, $P = 0.78$) or total fruit production by plants of the species in the EBC common garden ($r_s = 0.25$, $P = 0.59$) nor was total lifetime fruit production by EBC plants and CUCBA plants correlated ($r_s = 0.68$, $P = 0.16$; Fig. 3a).

The total dry weight of the EBC plants differed by species ($F = 4.76$; $df = 9, 38$; $P = 0.0003$; Table 6). The dry weight of a single plant ranged from 4.56 ± 0.96 g (*P. solanaceous*) to 20.41 ± 1.42 g (*P. nicandroides*). The *Physalis* species differed in the percent of the total mass of EBC common garden plants that was accounted for by the roots ($F = 8.45$; $df = 9, 38$; $P < 0.0001$), stems ($F = 3.97$; $df = 9, 38$; $P < 0.002$), leaves ($F = 5.98$; $df = 9, 38$; $P < 0.0001$), and fruits ($F = 10.40$; $df = 9, 38$; $P < 0.0001$).

Physalis species had a significant effect on diameter (CUCBA: $df = 6, 188$, $F = 20.13$, $P < 0.0001$; EBC: $df = 9, 406$; $F = 76.37$; $P < 0.0001$; MPICE: $df = 9, 202$, $F = 106.69$, $P < 0.0001$; Table 6). Diameters of EBC fruits were positively correlated to the diameters of CUCBA fruits ($r = 0.89$, $P = 0.02$) and to the diameters of MPICE fruits ($r = 0.87$, $P = 0.003$; Fig. 3b).

For EBC plants, the species differed in the number of seeds per fruit ($F = 19.12$, $df = 9, 89$; $P < 0.0001$; Table 6). *Physalis pruinosa* had the most seeds per fruit (121.2 ± 19.6 seeds), and *P. solanaceous* had the fewest seeds per fruit (6.3 ± 0.68 seeds).

Seed weight differed by species ($F = 302.65$, $df = 8, 351$; $P < 0.0001$; Table 6). The species with the heaviest seeds was *P. nicandroides* (28.0 ± 0.6 mg). The species with the lightest seeds were *P. cordata* (3.4 ± 0.2 mg), *P. microcarpa* (3.5 ± 0.2 mg), and *P. minuta* (3.4 ± 0.2 mg).

Trade-offs among life history characteristics

Using the data from the EBC common garden study, we conducted principal component analyses on the life history characteristics (Tables 7 and 8). Three principal components accounted for 84.6% of the total variance, indicating that life history traits are interrelated. The number of fruit produced per plant was negatively correlated with the number of seeds per fruit and the diameter of the fruits. Variation in these traits was closely aligned with the first principal component (PC1) which explained 45.77% of the variance. Thus, fruit production by the study species fell along a continuum, with species like *P. pruinosa* (PC1 = 3.08; in the EBC a mean of 20.1 ± 1.12 fruits produced per plant and 121.2 ± 19.6 seeds per fruit) at one end of the scale and species like *P. solanaceous* (PC1 = -2.94; in the EBC a mean of 156.0 ± 1.19 fruits produced per plant and 6.3 ± 0.68 seeds per fruit) at the other end of the scale. If the total number of fruit

produced per plant is multiplied by the number of seeds per fruit, then the number of seeds produced over the plants lifetimes does not differ by species (ln-transformed: $P = 0.15$).

A negative association between accumulation of total biomass and biomass allocation to the fruits was aligned with the second principle component (PC2), which accounted for 23.34% of the variance. This indicates that there is a trade-off between growth and reproduction. Variation in dry mass allocation to the leaves and seed weight was aligned with the third principle component (PC3), which represented 15.46% of the variance.

Relationship between life history and geographic distribution

The cosmopolitan species differed from the species with more narrow geographic ranges in that they had significantly higher PC1 scores (*i.e.* they produced fewer fruit with larger diameters and more seeds; Kruskal Wallis test Chi-square = 3.84, $P = 0.05$; Fig. 4a). The PC2 and PC3 scores did not differ significantly for cosmopolitan species versus species with more restricted geographic ranges. The species' PC1 scores were positively correlated with how common the species are in West-Central Mexico as indicated by the frequency with which they occurred at sites in West-Central Mexico ($r = 0.75$, $P = 0.02$; Fig. 4b). Likewise, PC1 scores for the species are positively correlated with the elevation gradient across which the species occur ($r = 0.68$, $P = 0.04$; Fig. 4c). Scores for PC2 and PC3 were not correlated with either the frequency with which the species occur in West-Central Mexico or the elevation gradient across which the species occurred ($P > 0.40$; Fig. 4d). The clusters of co-occurring species did not differ significantly with respect to PC1, PC2, or PC3, indicating that success of a species in a specific habitat did not require specific life history traits.

DISCUSSION

Within this group of closely related plants, trade-offs in the species' life history characteristics result in contrasting life history strategies. Some species tend to produce fewer, larger fruit with more seeds while other species produce many small fruits with few seeds. Plants of some species invest more in the accumulation of biomass (growth) but sacrifice allocation of biomass to fruits. Common and widespread species tended to produce fewer, larger fruits with more seeds per fruit. In similar studies on plant taxa with contrasting geographic distributions, species with more limited geographic ranges often produced fewer seeds per fruit (Byers and Meagher 1997, Jakobsson and Eriksson 2000, Lavergne *et al.* 2004).

Although fruit size and fruit production are interrelated, intrinsic differences in the size of fruits produced by a species may have more power to explain differences in species'

biogeographies than differences in productivity. For a given species, the relative sizes of fruits produced by plants were more stable across environments than fruit productivity across environments. The species that produced large, many-seeded fruits are more widespread geographically. *Physalis* fruits match the stereotypical “mammalian dispersed” seed syndrome (Van der Pijl 1982). Smaller fruit may be less attractive to vertebrate seed dispersers because they have less reward (as measured by sheer size), especially when compared to the ratio of the weight of the fruit to the weight of the calyx. Likewise, the handling time may be higher for smaller fruit. Seed dispersal by vertebrate agents is particularly advantageous for species like *Physalis* that grow in unpredictable, ruderal, or xeric environments (Wenny 2001, Bolmgren and Eriksson 2005). Alternatively, the pulp of some fleshy fruits has been shown to have properties that protect seeds from environmental hazards (Cipollini and Levey 1997). Thus larger, pulpier fruits may be an adaptation to cope with variable environments. Future studies should examine trade-offs in the continuum between producing many small fruits with few seeds versus few large fruits with more seeds.

The quest to identify overarching trends in the relationship between plant life histories and environmental factors has not been conclusive (Franco and Silvertown, 1996). While some species may converge on similar responses to environmental selection pressures, there does not seem to be only one adaptive syndrome for coping with a specific environmental stressor. Such appears to be the case for *Physalis* species. None of the life history characteristics evaluated in this study were significant predictors of species co-occurrence. In fact, the two sets of co-occurring species include representatives of opposing ends of the spectrum with respect to the growth versus reproduction trade-off and the seed packaging trade-off.

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Table 1. Biogeography of the eleven *Physalis* species examined in the field and in a common garden in West-Central Mexico.

<i>Physalis</i> species	Geographic range ¹	Ecoregion ²	Elevational gradient (Max-Min m) ³	Frequency of occupied sites in West-Central Mexico ³	Co-occurrence ³	Field	Common garden	
							CUCBA	EBC
<i>P. ampla</i>	Central Mexico	Mesoamerican and Sierra Madres conifer-oak forests, Southern Mexican dry forests	1,244	30	<i>P. angulata</i> , <i>P. lagascae</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i> , <i>P. pruinosa</i> , <i>P. pubescens</i>	X	X	X
<i>P. angulata</i>	North and South America; introduced old World Tropics	Central Mexican matorral, Southern Mexican dry forest, Mesoamerican and Sierra Madre conifer-oak forests	1,315	22	<i>P. ampla</i> , <i>P. lagascae</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i> , <i>P. pruinosa</i> , <i>P. pubescens</i>	X	X	X
<i>P. cordata</i>	North and South America; introduced old World Tropics	Mesoamerican conifer-oak forest, Southern Mexican dry forest, South Pacific Coast mangrove & palm	1,848	18	<i>P. leptophylla</i> , <i>P. minuta</i>	X	.	X
<i>P. lagascae</i>	Mexico, Central America, and the Antilles	Central Mexican matorral, Southern Mexican dry forest, South Pacific Coast mangrove, Mesoamerican and Sierra Madre conifer-oak forests	1,921	17	<i>P. ampla</i> , <i>P. angulata</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i> , <i>P. pruinosa</i> , <i>P. pubescens</i>	X	X	X
<i>P. microcarpa</i>	Mexico, Central America, and the Antilles	Southern Mexican dry forest, Trans-Mexican Volcanic Belt forest	514	3	<i>P. ampla</i> , <i>P. angulata</i> , <i>P. lagascae</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i> , <i>P. pruinosa</i> , <i>P. pubescens</i>	.	.	X
<i>P. minuta</i>	Mexico and Central America	Southern Mexican dry forest	27	9	<i>P. cordata</i> , <i>P. leptophylla</i>	X	X	X
<i>P. nicandroides</i>	Mexico and Central America	Central Mexican matorral, Southern Mexican dry forest, Mesoamerican and Sierra Madre conifer-oak forests	1,525	30	<i>P. ampla</i> , <i>P. angulata</i> , <i>P. lagascae</i> , <i>P. philadelphica</i> , <i>P. pruinosa</i> , <i>P. pubescens</i>	X	X	X
<i>P. philadelphica</i>	Mexico, introduced US and Central America	Central Mexican matorral, Southern Mexican dry forest, Mesoamerican and Sierra Madre conifer-oak forests	.	38	<i>P. ampla</i> , <i>P. angulata</i> , <i>P. lagascae</i> , <i>P. nicandroides</i> , <i>P. pruinosa</i> , <i>P. pubescens</i>	X	X	X
<i>P. pruinosa</i>	North and South America	Southern Mexican dry forest, Mesoamerican and Sierra Madre conifer-oak forests	1,815	16	<i>P. ampla</i> , <i>P. angulata</i> , <i>P. lagascae</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i> , <i>P. pubescens</i>	X	.	X
<i>P. pubescens</i>	North and South America; introduced old World Tropics	Central Mexican matorral, Mesoamerican and Sierra Madre conifer-oak forests, Southern Mexican dry forests	1,925	21	<i>P. ampla</i> , <i>P. angulata</i> , <i>P. lagascae</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i> , <i>P. pruinosa</i>	X	X	.
<i>P. solanaceous</i>	Southern US and Mexico	Central Mexican matorral	715	4	<i>P. cinerascens</i> , <i>P. hastatula</i> , <i>P. patula</i>	X	.	X

¹. Vargas *et al.* 2003, ². Olson and Dinerstein 1998, ³. Chapter 3.

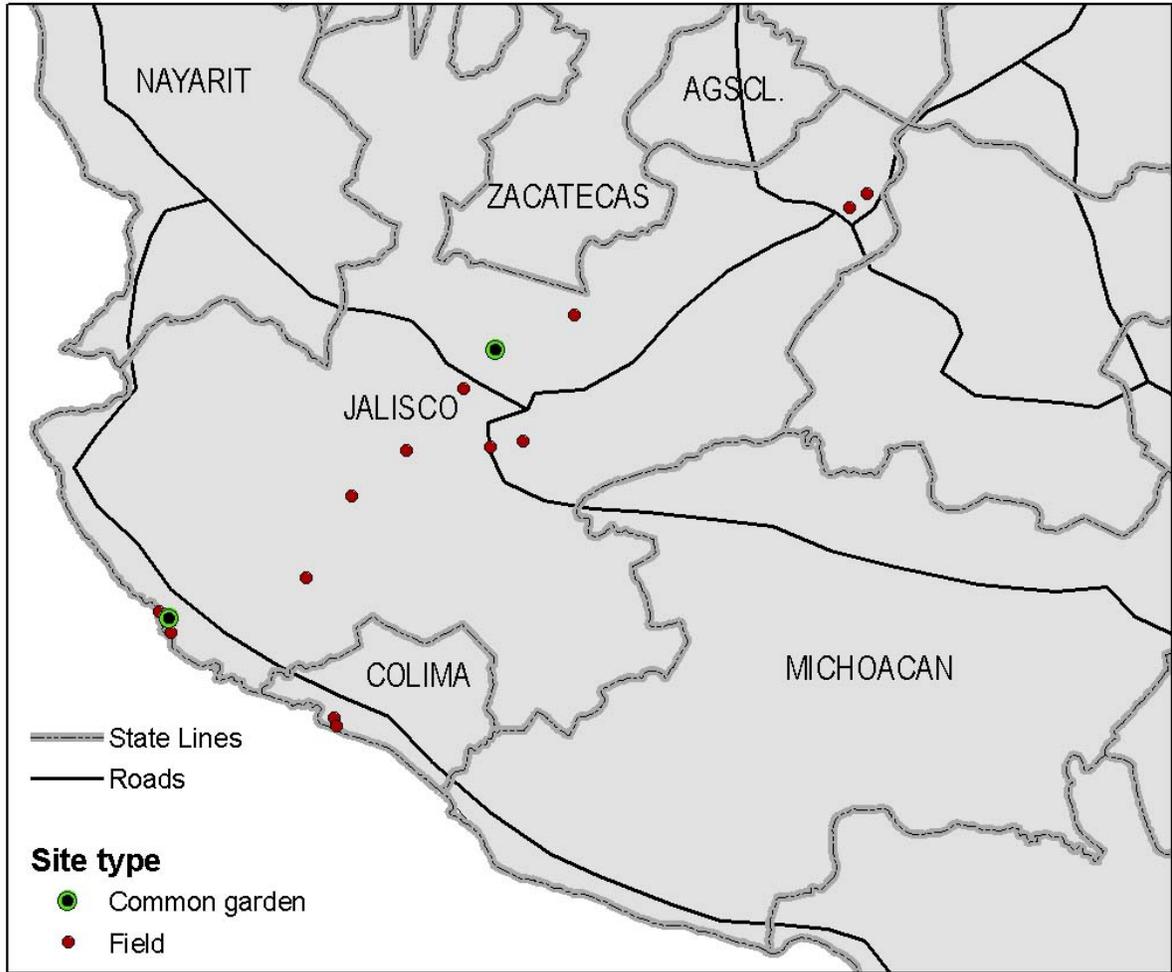


Fig. 1. Map of the 15 field sites and the two common garden sites in West-Central Mexico.

Table 2. Description of field sites in West-Central Mexico at which *Physalis* phenology was followed.

State	Municipality	Location	Coordinates	Ecoregion ¹	Elevation (m)	Description	Dates of data collection	<i>Physalis</i> species (N)
Jalisco	La Huerta	Xametla	-105° 5' 6" W, 19° 32' 14" N	PC	10	Dunes	8/11/04, 9/2/04, 9/29/04, 10/14/04	<i>P. minuta</i> (114)
Jalisco	La Huerta	Careyitos	-105° 1' 34" W, 19° 26' 16" N	PC	10	Under thorn bush on margin of beach	8/11/04, 9/2/04, 9/29/04, 10/13/04	<i>P. minuta</i> (99)
Colima	Manzanillo	Manzanillo	-104° 15' 10" W, 19° 1' 59" N	PC	13	Near mangrove swamp	8/11/03, 8/31/04, 9/21/04, 10/15/04	<i>P. lagascae</i> (100)
Colima	Manzanillo	Manzanillo	-104° 14' 25" W, 18° 59' 40" N	PC	29	Dunes	9/29/02, 6/16/03, 8/31/03, 10/4/03, 8/31/04, 9/21/04, 10/15/04, 11/4/04	<i>P. minuta</i> (114)
Jalisco	La Huerta	Arroyo Chamela	-105° 2' 57" W, 19° 31' 27" N	SMDF	34	Along dry river bed, under thorn bushes	7/12/03, 7/26/03, 8/11/04, 9/2/04, 9/23/04, 10/14/04	<i>P. cordata</i> (107), <i>P. pruinosa</i> (22), <i>P. pubescens</i> (8)
Jalisco	Autlan	Los Mazos	-104° 23' 1" W, 19° 41' 46" N	SMDF	1,292	Roadside	10/23/02, 7/27/03, 9/9/04, 9/29/04, 10/9/04	<i>P. ampla</i> (.), <i>P. angulata</i> (6), <i>P. cordata</i> (11), <i>P. lagascae</i> (12), <i>P. nicandroides</i> (2), <i>P. philadelphica</i> (6)
Jalisco	Tenmaxtlan	Colotitlan	-104° 10' 0" W, 20° 5' 0" N	SMDF	1,396	At edge of cornfield	10/23/02, 9/9/04, 9/29/04, 10/9/04	<i>P. angulata</i> (1), <i>P. lagascae</i> (57), <i>P. nicandroides</i> (4)
Jalisco	Tala	Bosque Escuela la Primavera	-103° 38' 18" W, 20° 35' 49" N	TMVB	1,472	In pasture and forest's edge	7/17/03, 8/20/03, 9/21/03, 11/4/03	<i>P. ampla</i> (18), <i>P. lagascae</i> (2), <i>P. nicandroides</i> (46), <i>P. pruinosa</i> (6), <i>P. pubescens</i> (3)
Jalisco	Zapopan	C.U.C.B.A., Universidad de Guadalajara	-103° 30' 0" W, 20° 47' 0" N	SMDF	1,600	Edge of cornfields	9/26/02, 10/8/02, 10/15/02, 11/13/02, 7/7/03	<i>P. ampla</i> (11), <i>P. nicandroides</i> (1), <i>P. philadelphica</i> (25), <i>P. pubescens</i> (2)
Jalisco	Jocotepec	Barranca de Huariche	-103° 30' 24" W, 20° 18' 55" N	TMVB	1,683	Corn field on rocky slope	9/3/03, 9/17/03, 10/12/03	<i>P. ampla</i> (7), <i>P. angulata</i> (6), <i>P. lagascae</i> (9), <i>P. nicandroides</i> (28), <i>P. pruinosa</i> (18), <i>P. pubescens</i>
Jalisco	Cocula	Arroyo Colorado	-103° 55' 30" W, 20° 18' 6" N	TMVB	1,706	In corn field and under trees at field's edges	10/23/02, 7/27/03, 9/9/04, 9/29/04, 10/9/04	<i>P. ampla</i> (46), <i>P. nicandroides</i> (25), <i>P. philadelphica</i> (59), <i>P. pruinosa</i> (13)
Jalisco	Jocotepec	Between Los Trojes and El Potrillo	-103° 21' 3" W, 20° 20' 45" N	TMVB	1,756	Along irrigation ditch at edge of corn field	9/3/03, 9/17/03, 10/12/03	<i>P. ampla</i> (22), <i>P. angulata</i> (33), <i>P. lagascae</i> (11), <i>P. philadelphica</i> (3), <i>P. pubescens</i> (32)
Jalisco	Ixtlahuacan del Rio	El Coyotillo	-103° 6' 31" W, 20° 56' 45" N	OSM	1,824	Tomatillo field and along road	7/28/03, 8/19/03, 9/27/03, 11/22/03, 8/4/04, 9/7/04	<i>P. ampla</i> (7), <i>P. angulata</i> (17), <i>P. nicandroides</i> (3), <i>P. pubescens</i> (21)
Jalisco	Lagos de Moreno	Paso de Cuarenta	-101° 47' 59" W, 21° 27' 19" N	CMM	1,931	In pasture under bushes	8/6-8/7/03, 11/1/03, 8/15/04, 9/6/04, 10/16/04	<i>P. angulata</i> (64), <i>P. nicandroides</i> (13), <i>P. pubescens</i> (13), <i>P. solanaceous</i> (59)
Jalisco	Lagos de Moreno	Rancho el Roble	-101° 43' 7" W, 21° 31' 16" N	CMM	1,973	Along road	8/6/03, 8/15/04, 9/6/04, 10/16/04	<i>P. angulata</i> (35)

¹. Pacific Coast (PC), Southern Mexican Dry Forest (SMDF), Trans-Mexican Volcanic Belt (TMVB), Occidental Sierra Madre (OSM), Central Mexican Matorral (CMM)

Table 3. (A.) Analysis of variance on fruit production by plants of *Physalis* species over time (relative to calendar date for field plants and plant age for common garden plants). (B.) Results on analysis of variance on total lifetime fruit production by CUCBA plants.

A.

Effect	Field				CUCBA ¹				EBC ²			
	df	MS	F- value	P	df	MS	F- value	P	df	MS	F- value	P
Whole model												
Model	29	23.05	18.04	<0.0001	13	39.3	35.63	<0.0001	29	30.67	44.05	<0.0001
Error	1292	1.28			882	1.1			427	0.7		
Model components												
Species	9	4.22	3.3	0.0005	6	5.75	5.21	<0.0001	9	6.46	9.28	<0.0001
Date	1	0.5	0.39	0.53	1	288.61	261.63	<0.0001	1	97.82	140.51	<0.0001
Species X Date	9	4.27	3.34	0.0005	6	7.66	6.94	<0.0001	9	7.5	10.77	<0.0001
Date X Date	1	0.0007	0.0007	0.98	-	-	-	-	1	56.42	81.05	<0.0001
Species X Date X Date	9	4.25	3.3	0.0005	-	-	-	-	9	6.94	9.96	<0.0001

B.

Effect	df	MS	F- value	P
Whole model				
Model	52	2.08	2.18	0.001
Error	72	0.95		
Corrected total	124	176.61		
Model components				
Species	6	6.18	6.48	<0.0001
Block	7	1.74	1.83	0.09
Species x Block	39	1.5	1.58	0.05

1. Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA) of the Universidad de Guadalajara, Zapopán, Jalisco, Mexico. ². Estación de Biología Chamela, Universidad Nacional Autónoma de México, Chamela, Jalisco, Mexico.

Table 4. Comparison of the least squares means of the numbers of fruits per senescent field plant and the means of lifetime fruit production by plants grown in two common gardens for 11 *Physalis* species (± 1 SE). Means with the same letter are not significantly different at $P = 0.05$.

<i>Physalis</i> species	FIELD	CUCBA	EBC
<i>P. ampla</i>	16.6 \pm 0.3 ab	51.9 \pm 12.9 a	72.4 \pm 11.4 ab
<i>P. angulata</i>	16.6 \pm 0.3 ab	6.9 \pm 1.6 b	29.2 \pm 10.8
<i>P. cordata</i>	3.71 \pm 0.3 c	.	5.0 \pm 3.5 b
<i>P. lagascae</i>	7.8 \pm 0.2 bc	18.2 \pm 7.2 ab	53.3 \pm 37.7 ab
<i>P. microcarpa</i>	.	.	100.4 \pm 40.9 ab
<i>P. minuta</i>	3.5 \pm 0.2 c	27.4 \pm 6.7 ab	117.7 \pm 28.8 ab
<i>P. nicandroides</i>	12.6 \pm 0.3 b	24.8 \pm 6.5 ab	43.6 \pm 10.6 ab
<i>P. philadelphica</i>	15.9 \pm 0.2 b	24.6 \pm 5.9 ab	23.2 \pm 12.3 b
<i>P. pruinosa</i>	18.5 \pm 0.6 ab	.	20.5 \pm 2.6 b
<i>P. pubescens</i>	19.3 \pm 0.4 ab	36.6 \pm 6.6 a	.
<i>P. solanaceous</i>	72.7 \pm 0.5 a	.	156.0 \pm 26.8 a

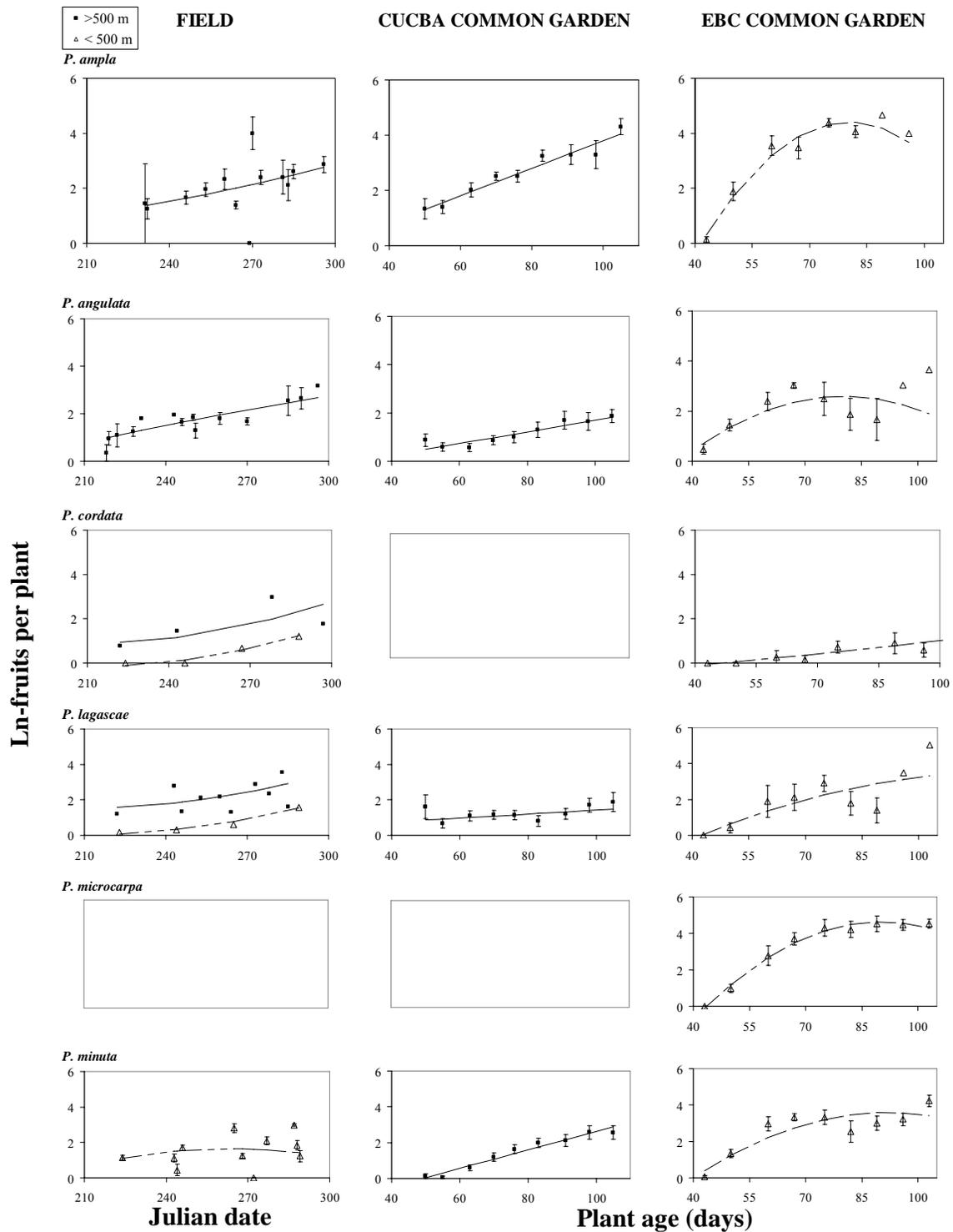


Fig. 2. Phenology of fruiting of plants of 11 *Physalis* species (rows) in the field and in two common gardens (columns). Mean fruits per plant calculated separately for field sites above and below 500 m in elevation.

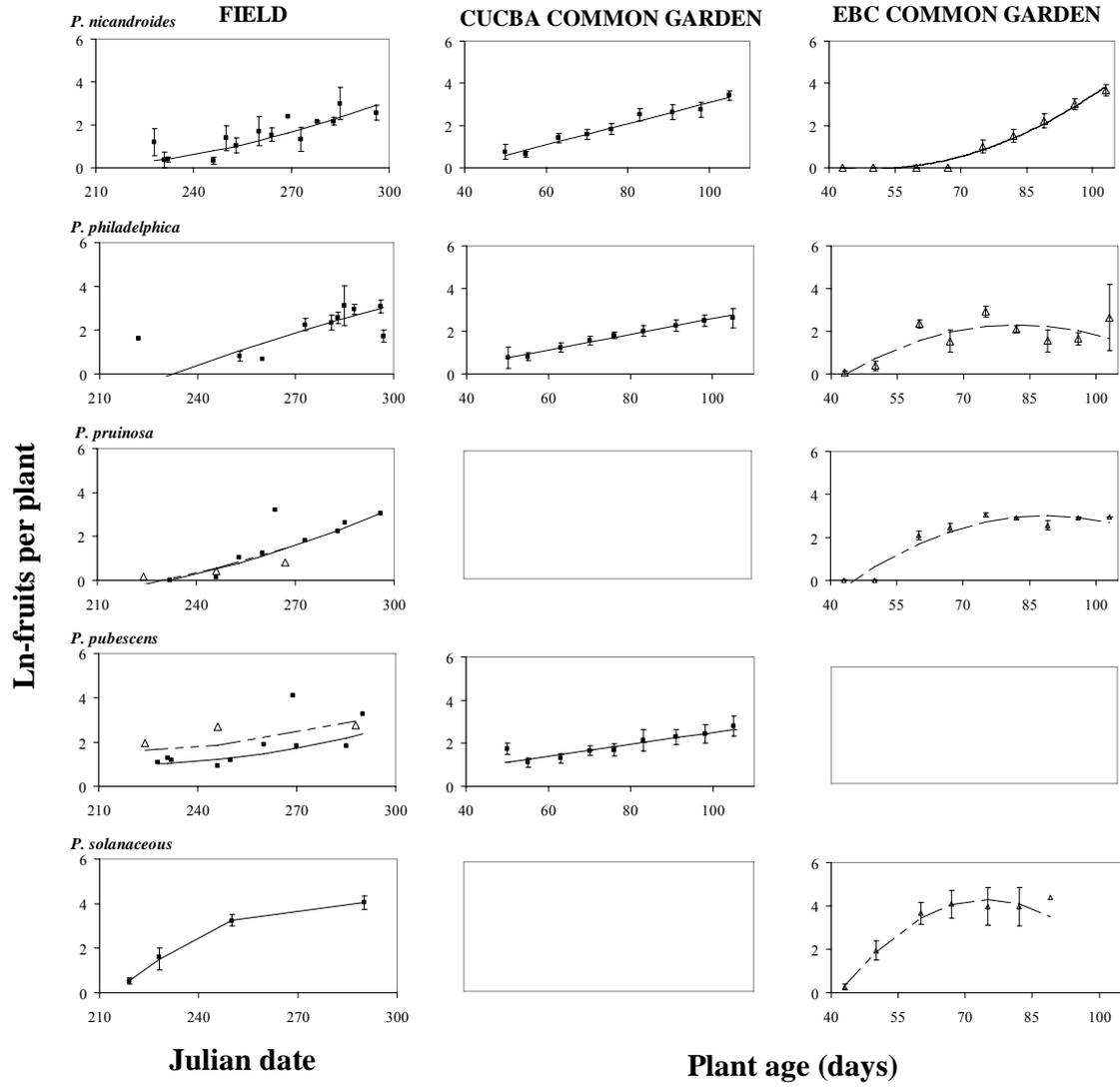


Fig. 2. (continued).

Table 5. Analysis of variance on the number of fruits associated with plants over time (relative to calendar date) for four *Physalis* species that occurred at sites from sea level to over 1,800 m in elevation over time. The sites are categorized by elevation (below or above 500 m).

Effect	df	MS	F- value	P
Model	12	29.24	0.3384	<0.0001
Error	430	0.86		
Elevation	1	9.46	10.95	0.001
Species	3	2.81	3.25	0.02
Elevation X Species	3	13.59	15.72	<0.0001
Date	1	2.07	2.4	0.12
Species X Date	3	2.41	2.79	0.04
Date X Date	1	3.49	4.04	0.05

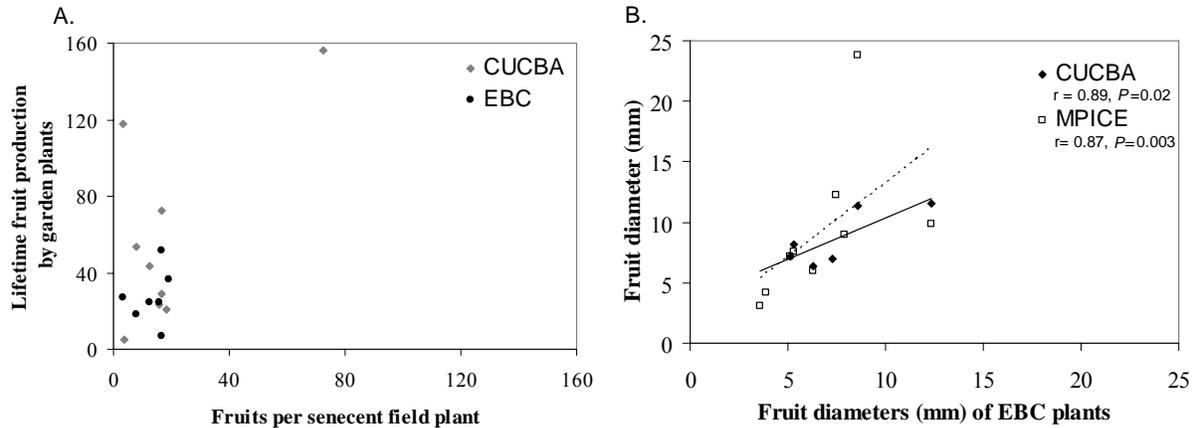


Fig. 3. Comparison of (A.) the number of fruit per senescent field plant of a species and lifetime fruit production by common garden plants of the species and (B.) fruit diameters for plants of a species reared in two common gardens (CUCBA and EBC) and the greenhouse (MPICE).

Table 6. Mean (± 1 SE) values of life history characteristics of EBC common garden plants. Means with the same letter are not significantly different.

Species	Productivity	Fruit characteristics			Biomass allocation				
	Lifetime fruit production	Fruit diameter (mm)	No. seeds per fruit	Seed weight (g)	Total dry weight (g)	Percent allocated to roots	Percent allocated to stems	Percent allocated to leaves	Percent allocated to fruits
<i>P. ampla</i>	72.4 \pm 11.4	1.07 \pm 0.03 f	20.6 \pm 3 d	.	7.89 \pm 0.72 bc	7.31 \pm 11.38 c	27.61 \pm 4.79 bc	26.49 \pm 4.12 c	338.60 \pm 7.40 c
<i>P. angulata</i>	29.2 \pm 10.8	1.56 \pm 0.06 c	47.6 \pm 6.57 c	1.2 c	11.32 \pm 0.8 abc	8.94 \pm 10.8 c	30.42 \pm 2.55 abc	24.40 \pm 2.26 bc	36.24 \pm 5.09 bc
<i>P. cordata</i>	5.0 \pm 3.5	1.65 \pm 0.13 bc	90.3 \pm 17.1 ab	3.4 f	18.23 \pm 4.59 ab	32.37 \pm 3.51 a	34.31 \pm 7.41 abc	32.07 \pm 4.89 bc	1.25 \pm 1.23 bc
<i>P. lagascae</i>	53.3 \pm 37.7	1.52 \pm 0.05 cd	57.4 \pm 4.06 bc	4.5 e	12 \pm 3.88 abc	8.89 \pm 37.71 c	43.90 \pm 6.28 ab	31.88 \pm 5.44 ab	15.33 \pm 4.84 ab
<i>P. microcarpa</i>	100.4 \pm 40.9	0.82 \pm 0.03 g	13.9 \pm 1.37 d	3.5 f	6.91 \pm 2.62 bc	9.27 \pm 40.89 c	33.15 \pm 5.79 abc	42.22 \pm 7.21 bc	15.37 \pm 5.06 bc
<i>P. minuta</i>	117.7 \pm 28.8	1.31 \pm 0.03 de	45 \pm 6.32 c	3.4 f	17.31 \pm 2.57 ab	17 \pm 28.84 bc	34.37 \pm 3.49 abc	27.77 \pm 2.94 bc	20.87 \pm 3.80 bc
<i>P. nicandroides</i>	43.6 \pm 10.6	1.12 \pm 0.06 ef	28 \pm 7.06 d	28.0 a	20.41 \pm 1.42 a	36.25 \pm 10.61 a	34.51 \pm 3.42 abc	17.28 \pm 2.68 bc	11.96 \pm 4.49 bc
<i>P. philadelphica</i>	23.2 \pm 12.3	1.8 \pm 0.23 b	56 \pm 14.57 c	17.0 b	16.63 \pm 1.63 ab	4.85 \pm 12.27 c	18.95 \pm 3.40 c	14.26 \pm 4.14 c	61.94 \pm 7.88 c
<i>P. pruinosa</i>	20.5 \pm 2.6	2.59 \pm 0.08 a	121.2 \pm 19.61 a	18.0 b	13.28 \pm 3.01 abc	15.74 \pm 2.6 bc	29.87 \pm 3.65 abc	7.61 \pm 2.65 c	46.78 \pm 7.68 c
<i>P. solanaceous</i>	156.0 \pm 26.8	0.75 \pm 0.04 g	6.3 \pm 0.68 e	7.6 d	4.56 \pm 0.96 c	10.31 \pm 26.76 c	50.51 \pm 3.71 a	14.74 \pm 3.16 a	24.44 \pm 4.98 a

Table 7. Correlation matrix of the 11 species' life history characteristics (following sequential Bonferroni correction: * $P = 0.10$, ** $P < 0.05$, *** $P < 0.001$).

Totalmass	Root percent	Stem percent	Leaves percent	Fruit percent	Seed weight	No. seeds/ fruit	Fruit diameter	Total fruit production	
	0.54	-0.006	-0.05	-0.35	0.10	0.55	0.54	-0.55	Totalmass
		0.50	0.09	-0.62	-0.05	0.07	-0.04	-0.08	Root percent
			0.26	-0.70	-0.27	-0.27	-0.49	0.48	Stem percent
				-0.68	-0.75 *	-0.14	-0.33	0.21	Leaves percent
					0.38	0.04	0.28	-0.05	Fruit percent
						0.08	0.25	-0.32	Seed weight
							0.94 ***	-0.83 *	No. seeds/fruit
								-0.87 **	Fruit diameter
									Total fruit production

Table 8. Eigenvectors of principal components analysis of life history characteristics of 11 *Physalis* species. Major relationships (factor loadings >0.35) are typed in boldface.

	PC 1	PC 2	PC 3
Totalmass	0.315	0.424	0.099
Root percent	0.094	0.620	0.274
Stem percent	-0.365	0.089	0.139
Leaves percent	-0.286	0.261	-0.501
Fruit percent	0.280	-0.554	0.066
Seed weight	0.309	-0.008	0.610
No. seeds	0.382	0.086	-0.374
Fruit diameter	0.438	-0.088	-0.275
Total fruit production	-0.407	-0.193	0.229

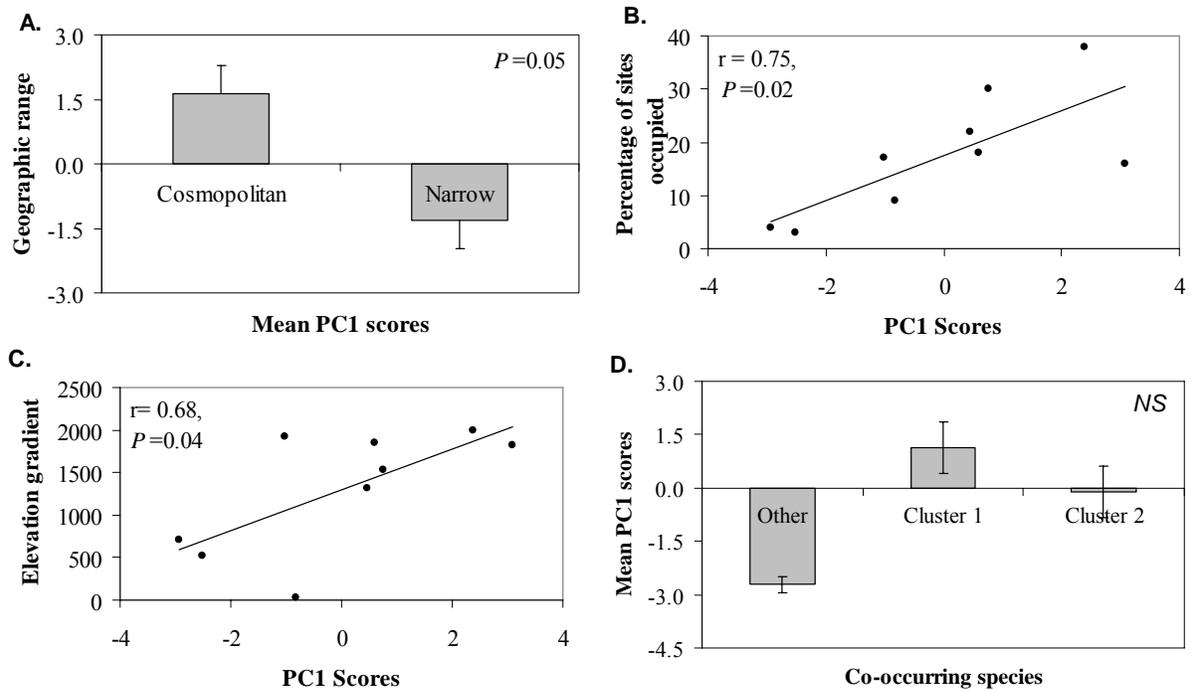


Fig. 4. Relationships between *Physalis* species scores for principal component 1 and the species' biogeographies. Species with high scores for PC1 produce fewer, larger fruit with more seeds per fruit) and tend to have (A.) larger geographic ranges, (B.) occur more frequently (C.) across a wide range of elevations than species with low scores for PC1. (D.) PC1 scores were not significantly different for clusters of species that tended to co-occur at field sites in West-Central Mexico.

5. VARIATION IN ESSENTIAL FATTY ACID CONCENTRATIONS IN THE
FRUITS OF NINE *PHYSALIS* SPECIES &
ITS RELATIONSHIP TO HERBIVORE DEFENSE

Abstract – Normal development of many insect species requires the acquisition of either linolenic acid, linoleic acid, or both through a dietary source. Variation in the concentrations of these two essential fatty acids in plant tissues may impact host use by phytophagous insects with a fatty acid requirement. It has been hypothesized that the genus *Physalis* L. (Solanaceae) has evolved to have low concentrations of essential fatty acids in their fruits as a defense against insect herbivores.

In the current study, we quantified the concentrations of essential fatty acids in fruits at different stages of maturation for nine *Physalis* species and two species from other Solanaceae genera. Both linolenic and linoleic acids were detected in fruits of all ages of all of the species but at different concentrations. The relationship between fruit age and fatty acid concentrations varied by species. This variation may be affected by the amount of anti-oxidant pigment compounds in the fruits of some *Physalis* species. The concentrations of linolenic and linoleic acids in *Physalis* fruits are not disproportionately lower than those found in fruits of species in other Solanaceae genera. These data do not support the hypothesis that the *Physalis* genus evolved lower levels of essential fatty acids as a defense against insect herbivores.

Key words – *Physalis*, *Heliothis subflexa*, Fruit maturation, Essential Fatty acids, Linolenic acid, Linoleic acid.

INTRODUCTION

For many insect herbivores, either one or both of the lipids linolenic acid and linoleic acid are essential nutrients that must be acquired through a dietary source (Nelson and Sukkestad 1968, Vanderzandt 1968, Dadd 1973, Tinoco 1982, Stanley et al. 1988). For those species with a dietary requirement for these two polyunsaturated fatty acids, deficiency can lead to slow growth, low pupal weights, malformed pupae, or mortality (Fraenkel and Blewett 1946, Chippendale et al. 1964, Nakasone and Ito 1967, Dadd 1973, Sivapalan and Gnanagragasm 1979). The requirement for polyunsaturated fatty acids is not the same for all insects (Thompson 1973). Some insects (Diptera in particular) can develop and reproduce normally on diets lacking both linolenic and linoleic acid (Dadd 1973). Meanwhile, normal development of most Lepidoptera and Hymenoptera specifically requires linolenic acid and not linoleic acid (Canavoso et al. 2001). Within the Lepidoptera, there are some exceptional species that are able to develop without linolenic acid (e.g. the Angoumois grain moth, *Sitotroga cerealella* [Oliv.], Chippendale 1971).

The fatty acid profiles of plants differ on a taxonomic basis, from tissue to tissue within an individual and by the age of the tissue (Whitaker 1986, Mongrand et al. 1998). The

concentrations of polyunsaturated fatty acids in plant tissues can vary by several orders of magnitude. For example, the concentration of linolenic acid ranges from as little as 0.50 mg/g in the potato to as high as 478.6 mg/g in the walnut (Tinoco 1982, Gebhardt and Thomas 2002). Fatty acids tend to be present at highest concentrations in seed oils (Tinoco 1982), but the concentration of lipids in fruit pulp tends to be very low (Herrera 1982). In many species it has been observed that metabolic processes associated with fruit maturation generate fatty acid peroxides which degrade polyunsaturated fatty acids (Brennen and Fraenkel 1977). In parallel to increases in oxidative stress, the activity of free-radical scavenging enzymes declines with fruit age (Kalra and Brooks 1973, Rogiers et al. 1998, Wang and Jiao 2001). Consequently, the saturation of fatty acids tends to increase as fruits mature (Galliard 1968, Kalra and Brooks 1973). Changes in the concentrations of essential fatty acids over the course of fruit maturation can be as great as or greater than differences between species (Rogiers et al. 1998).

Studies to date have found variation in the concentrations of fatty acids in plants in the *Physalis* L. (Solanaceae) genus. Upon analysis of *P. angulata* plants, De Moraes and Mescher (2004) found linolenic acid in the leaves and calyces but did not detect linolenic acid in the fruits. Linolenic acid is present in the fruits of the other *Physalis* species (e.g. *P. peruviana*, *P. pubescens*, and *P. ixocarpa*; Ramadan and Mörsel 2003, Deineka and Deineka 2004). The ages of the fruits that were analyzed were not standardized across studies. Thus, the variation in the concentrations of essential fatty acids may be due to interspecific differences within the *Physalis* genus, or it may be associated with unaccounted for differences in the maturity of the analyzed fruits.

Variation in the concentration of essential fatty acids in *Physalis* fruits may impact host use by insect herbivores. For example, caterpillars of *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) are extreme generalists capable of developing on plants in more than 14 different families (Sheck and Gould 1993), but *P. angulata* is not one of its hosts. De Moraes and Mescher (2004) demonstrated that a high proportion of the *H. virescens* caterpillars reared on untreated *P. angulata* fruits produced malformed pupae, which is a symptom of linolenic acid deficiency. When the *P. angulata* fruits were sprayed with linolenic acid supplement, the fraction of malformed pupae was significantly lower. In contrast to *H. virescens*, caterpillars of the taxonomically related species *Heliothis subflexa* G. feed exclusively on fruits of species in the *Physalis* genus, including *P. angulata*. Although not all *Physalis* species are suitable hosts for *H. subflexa*, *P. angulata* is one of its host species. *Heliothis subflexa*'s association with *P. angulata* fruits may indicate that this herbivore has adapted to survive on fruits with either no or low levels of linolenic and linoleic acids. The ability to develop normally in the absence of essential fatty

acids could confer benefits to this herbivore: linolenic acid is necessary for the production of volicitin, which is an elicitor of plant signalling compounds that attract parasitoids, and linolenic acid is a dietary requirement for parasitoid development. Based on the measurements of fatty acids in *P. angulata* fruits, it has been proposed that plants in the *Physalis* genus have evolved to have fruits that are deficient in essential fatty acids. Furthermore, De Moraes and Mescher (2004) have hypothesized that “*H. subflexa* larvae achieve multiple benefits from such narrow specialization: they gain access to a food resource that is unavailable to most of their competitors, co-opt the plants' physical and chemical deterrents for their own defense, render themselves nutritionally unattractive to parasitoids, and circumvent induced plant defenses elicited by volicitin, including the production of some volatiles attractive to natural enemies.”

The purpose of this study was two-fold. 1) We tested whether there were significant interspecific or ontogenetic differences in the concentrations of essential fatty acids in fruits of *Physalis* and of other species from other Solanaceae genera. 2.) We evaluated whether the *Physalis* genus has evolved to have fruits that are deficient in essential fatty acids as a defense against generalist insect herbivores. To address these two points, we quantified the concentrations of linolenic and linoleic acids in fruits representing different stages of maturation of nine *Physalis* species as well as two species from other Solanaceae genera (the tomato, which is a host of *H. virescens*, and *Nicandra physalodes*, which has a similar life history to that of species in the *Physalis* genus). We compared the linolenic and linoleic acid concentrations in other tissues of *P. angulata* (young and mature leaves, calyces of multiple ages, and stems).

METHODS

Study plants. We measured concentrations of linolenic and linoleic acids in fruits of nine *Physalis* species: *P. ampla*, *P. angulata*, *P. cordata*, *P. minuta*, *P. peruviana*, *P. philadelphica*, *P. pruinosa*, *P. pubescens*, and *P. solanaceous* (Table 1). *Heliothis subflexa* does not infest plants of *P. solanaceous*, but the other eight species are hosts of *H. subflexa*. Depending on the species and exposure to sunlight, mature fruits of these nine species can be green, yellow, orange, purple, or red in coloration (Vargas *et al.* 2003). This may reflect changes in the concentrations of antioxidants such as anthocyanins, flavonoids, or carotenoids over the course of maturation. For *P. angulata*, we measured the fatty acid concentration in fruits from two separate sources: a Mexican population grown in a greenhouse and a North Carolina population grown in a common garden. The North Carolina population comes from the same geographic area as the plants used in the study by De Moraes and Mescher (2004). The two *P. angulata* accessions are very different and may actually belong to two different species. We also evaluated fatty acids in the

fruits of the tomato (*Solanum lycopersicum* L. [Solanaceae]), which is a host of *H. virescens*, and *Nicandra physalodes* (Solanaceae), which has a life history similar to that of plants in the *Physalis* genus. Germplasm for the study plants came from field populations, previous common garden studies, germplasm banks, and commercial sources (Table 1). Voucher specimens were deposited in the herbarium of the Kew Royal Botanical Gardens.

With the exception of the North Carolina accession of *P. angulata*, all plants were grown in the greenhouses at the Max Planck Institute for Chemical Ecology, Jena, Germany between January 13th and August 12th of 2005. Seeds for the experiments were sown in four blocks (January 13th, February 18th, February 22nd, and March 9th). The plants were grown at 22±1°C, 55±5 Rh, and 14:10 light dark cycle. Plants did not experience drought, predator, or temperature stress, but some plants were infected with a pathogen. All plants exhibiting symptoms of pathogen infection were discarded.

Plants of the North Carolina accession of *P. angulata* were initially grown in seedling trays in a greenhouse at North Carolina State University, Raleigh, NC. When they were six weeks old, they were transplanted into a common garden at the Central Crops Research Station, Clayton, NC. There they experienced the prevailing conditions in the field: natural light, rainfall, and ambient temperature. Landscaping fabric limited the influence of competing plants, but the NC *P. angulata* did experience herbivore attack.

To class fruits by age, we followed one protocol for *Physalis* and *N. physalodes* plants (these species have similar life histories), and we classed tomato fruits by age using a different protocol (maturation of tomatoes is a more protracted process). For *Physalis* plants and for plants of *N. physalodes*, once an individual had a minimum of four flowers (37 – 71 days after seeds were sown, depending on the species), we marked four to 15 flowers with no signs of previous pollination (open flowers with erect petals, no pollen grains on the stigma, and no withering of the flower). Since some *Physalis* species are self-incompatible (Menzel 1951, Pandey 1957, Whitson and Manos 2005), we cross-pollinated flowers with pollen from conspecific plants in a random fashion (per Whitson 2001). Following the first round of hand pollination of each plant of *Physalis* or of *N. physalodes*, we marked four to 15 additional flowers every four days for a period of 12 days. For the tomato plants, we marked flowers on April 29th, 2005, but we did not mark any more flowers on subsequent dates.

Harvests of the greenhouse plants were carried out in a separate laboratory to prevent induction of other plants. Fruit from the North Carolina accession of *P. angulata* were harvested in the field. All of the fruits of each individual plant were harvested at the same time. Due to abscission of some flowers and fruits, not all marked inflorescences produced fruit. Harvests of

the *Physalis* plants and the *N. physalodes* plants were staggered such that the fruits collected represented the whole process of fruit maturation from 4 to 31 days of age. Maturation of tomato fruit is a more protracted process. The oldest harvested tomato fruits were between 94 and 105 days old. From those same plants, we also harvested green, immature tomatoes. The exact age in days of the unripe tomato fruits is not known. The diameters of each of the harvested fruits of the plants of the nine *Physalis* species, *N. physalodes*, and the tomato were measured from the point of attachment to the peduncle to the abscission scar of the flower.

We collected leaves, stems, and calyces of the Mexican accession of *P. angulata*. We classed *P. angulata* calyces and leaves by age. Calyx age was the same as the age of the fruit to which it was attached. Young leaves were collected from terminal rosettes. They were pale green in color, semi-translucent, pliable, and not fully expanded. Mature leaves were collected from lateral nodes. They were dark green in color, more opaque, tougher, and fully expanded. We did not attempt to take into account the age of the pieces of stem.

As plant material was collected, it was stored on ice. We pooled the harvested material of each individual by tissue type and age into aliquots (up to 0.5 g fresh weight). We recorded the fresh weight of the sample. We lyophilized the material for 24 hours and recorded the dry weight of each sample. We calculated the percent water content of each sample. Prior to extraction, we stored the plant material at -20°C.

Fatty acid extractions. Fatty acids were transesterified into fatty acid methyl esters following the procedure described by Rodríguez-Ruiz (1998). Using a high performance dispenser (Polytron PT 1300 D, Kinematica AG, Lucerne, Switzerland) we homogenized each sample for approximately 90 seconds in a solution of 1 ml of acetyl chloride and methanol (1:19, v/v) with 15 µg of heptadecanoic acid (which does not occur in nature) as an internal standard. Once the samples were thoroughly macerated, we added 1 ml of hexane. We mixed and heated the hexane: acetyl chloride: MeOH solution at 100°C for half an hour. We cooled the solution to room temperature and collected the hexane phase with the total fatty acid methylesters. To standardize the volume of hexane of each sample, we evaporated the hexane under a gentle stream of nitrogen gas and then added 1 mL of hexane to each vial.

The fatty acid methyl esters were analyzed using an iontrap GC-MS (Finnigan GCQ, Thermoelectron, Bremen, Germany) equipped with an Alltech EC-5 capillary column (30 m x 0.25 mm x 0.25 µm (Alltech, Uchtenhagen, Germany). 1 µl of each sample was automatically injected with a split ratio of 1:10, the injector was held at 260 °C, the transferline was kept at 270 °C. Separation of compounds was achieved under programmed conditions from 60 °C (with a 1

min hold) to 180 °C at 10 °C/min followed by 4 °C/min to 280 °C and held constant for 1 min.. A subset of samples were also analyzed using a 30 m x 0.25 mm x 0.25 µm DB225 column (J & W Scientific, Folsom, CA, USA). To confirm the identity of each fatty acid peak, we compared its retention time to the retention time of an authentic standard fatty acid methyl ester mixture (FAME mixture, Supelco, Bellefonte, PA, USA), and we used mass spectrometry to scan the samples for characteristic ions of each of the fatty acid methyl esters. Quantification of the essential fatty acid methyl esters was based on the total count of the characteristic ions 294 (linoleic acid) and 292 (linolenic acid) relative to recovery of the heptadecanoic internal standard (Dodds et al. 2005). We estimated the fatty acid concentrations in the plant samples on a per weight basis. We measured calibration curves adding increasing amounts of linolenic acid (5 - 40 µg) to 15 µg of the internal standard heptadecanoic acid and methylated these samples with diazomethane. We plotted the peak area ratio of the linolenic acid and the heptadecanoic acid against the added amount of linolenic and linoleic acids.

Statistical analyses. For the *Physalis* species and for *N. physalodes*, we used mixed model analysis of variance (PROC MIXED, SAS) to test whether fruit diameter, percent water content, or the concentrations of linolenic and linoleic acids differed by the plant species fixed effect, the linear effect of fruit age, the quadratic effect of fruit age, or any of the interactions between plant species and fruit age. The plant individual nested in plant species was included in the model as a random effect (Appendix C for the SAS code). Whenever the overall test for equality among species was significant at 0.05, post hoc comparisons using *pdiff* and Tukey adjustment were carried out among the *Physalis* species and the control species *N. physalodes*. Using t-tests, we compared the fruit diameters, percent water content, and the concentrations of linolenic and linoleic acids in ripe (red) and unripe (green) tomato fruits. In all analyses fruit age was log-transformed, percent water content was arcsine-square root transformed, and the concentrations of essential fatty acids were log-transformed.

We used mixed model analysis of variance to assess whether the concentrations of essential fatty acids in *P. angulata* (Mexican accession) differed by tissue type (fruits, calyces, young leaves, mature leaves, and stems). Plant individual nested in species was included in the model as a random effect.

All statistical analyses were conducted using SAS software (SAS 8.2 for Windows, SAS Institute, 2001). In all tests, the significance level was set at $\alpha=0.05$, and ± 1 standard error (SE) values are presented for estimated mean values.

RESULTS

Interspecific and ontogenetic differences in fruit diameter and water content. Fruit size differed by the linear effect of fruit age, the quadratic effect of fruit age, and all of their interactions (Tables 3 and 4, Fig. 1). After 25 days, *P. philadelphica* fruits were the largest (23.51 ± 0.74 mm), and *P. solanaceous* fruits were the smallest (3.00 ± 0.22 mm). The percent water content of the fruits varied significantly by *Physalis* species, fruit age, and the quadratic function of fruit age but not by any other interaction (Tables 3 and 4; Fig. 1). The percent water content of the fruits decreased by age, and fruits of some species lost water more precipitously than others. After 25 days, the percent water content of *Physalis* fruits ranged from $75.24\% \pm 0.02\%$ (*P. solanaceous*) to $92.84\% \pm 0.10\%$ (*P. pubescens*). Fruits of *N. physalodes* lost more water as they matured than fruits of any of the *Physalis* species. After 20 days, water accounted for $57.53\% \pm 0.02\%$ of the fresh weight of *N. physalodes* fruits. Tomato fruits increased in water content as they matured ($t=2.15$, $P=0.04$; unripe fruits= $92.19\% \pm 0.23\%$ and ripe fruits= $93.12\% \pm 0.40\%$).

Interspecific and ontogenetic variation in essential fatty acids. Both linolenic and linoleic acids were present in fruits of each study species. For *Physalis* and *N. physalodes* taken as a whole, the concentrations of linolenic and linoleic acids differed by plant species, the linear effect of fruit age, the quadratic effect of fruit age, and all of the interactions between plant species and fruit age (Tables 3 and 4, Fig. 1). The concentrations of essential fatty acids in *N. physalodes* fruits was not significantly different from the concentrations of essential fatty acids in fruits of several of the *Physalis* species, and concentrations in *N. physalodes* fruits were sometimes lower than in *Physalis* fruits. Likewise, the concentrations of essential fatty acids in tomato fruits were not dissimilar to the concentrations of essential fatty acids in *Physalis* fruits. Neither of these species from other Solanaceae genera had disproportionately higher levels of linolenic or linoleic acids.

The relationship between the concentration of linolenic acid and fruit age varied by species. Linolenic acid decreased linearly over time for three species (*P. ampla*, *P. angulata* [Mexican accession], and *P. cordata*). Linolenic acid concentrations varied curvilinearly over the course of fruit maturation for five species (*P. angulata* [NC], *P. pruinosa*, *P. pubescens*, *P. solanaceous*, and *N. physalodes*). There was no relationship between fruit age and linolenic acid concentration for two species (*P. peruviana* and *P. philadelphica*). Likewise, there were no differences in the concentrations of linolenic acid in ripe versus unripe tomato fruits ($P = 0.17$).

Linoleic acid varied curvilinearly by fruit age for four species (*P. minuta*, *P. philadelphica*, *P. pruinosa*, *P. pubescens*, and *N. physalodes*). There was no significant

relationship between linoleic acid concentration and fruit age for six species (*P. ampla*, *P. angulata* [NC & Mexico], *P. cordata*, *P. peruviana*, *P. solanaceous*).

The concentration of linolenic acid in fruits of *P. angulata* differed both by accession ($F=2003$, $P<0.0001$) and by the fruit age ($F=4.84$, $P=0.04$), but not by any of the other interactions. Common garden fruits of the NC accession have lower linolenic acid concentrations than the greenhouse fruits of the Mexican accession. The concentration of linoleic acid in fruits of *P. angulata* was not affected by accession, fruit age, or any of the other interactions ($P>0.10$).

Variation by P. angulata plant tissue. Concentrations of linolenic acid varied significantly by plant tissue (df=4, 60, $F=24.24$, $P<0.0001$; Fig. 2). The concentration of linolenic acid was highest in the leaves (2.92 ± 0.80 mg/g fresh weight of young leaves and 3.06 ± 0.57 mg/g fresh weight mature leaves) and lowest in the fruits (0.44 ± 0.11 mg/g fresh weight). Linoleic acid did not differ significantly by tissue type (df=4,57, $F=0.98$, $P=0.43$). Neither the concentrations of linolenic nor linoleic acids were significantly related to calyx age (Linolenic acid: $r=0.23$, $P=0.32$; Linoleic acid: $r=-0.12$, $P=0.60$; Fig. 4).

DISCUSSION

Both linolenic and linoleic acid were present in fruits of all of the study species, but the concentrations differed by species and fruit age. Likewise, both of these fatty acids were present in the other plant tissues that were examined. The *Physalis* genus as a whole does not appear to have evolved significantly lower levels of essential fatty acids. There were no marked differences in the concentrations of essential fatty acids separating the *Physalis* genus from the other two species from other Solanaceae genera. The concentration of linolenic and linoleic acids in fruits of tomatoes and of *N. physalodes* was comparable to that of many of the *Physalis* species. The fact that the concentrations of essential fatty acids in the fruits of some *Physalis* species actually exceeds the concentrations essential fatty acids in tomato fruits suggests that nutrient deficiency alone can not explain why *Physalis* species are not hosts of *H. virescens*. De Moraes and Mescher (2004) observed a decrease in the production of malformed pupae when *H. virescens* caterpillars were reared on *P. angulata* fruits that had been sprayed with linolenic acid supplement. Similar findings have been found for *Helicoverpa zea* feeding on diets containing undecanone (Farrar *et al.* 2002). The addition of undecanone to the diet resulted in malformed pupae, but insects reared on diet with both undecanone and linolenic acid supplement produced normal pupae. Thus, *Physalis* plants may contain compounds such as undecanone which make linolenic acid less available to the insect.

In a companion study, we found that the concentrations of four saturated fatty acids declined with age, but the concentration of oleic acid was not affected by fruit age (Bateman et al. in preparation). Much of the ontogenetic variation in fatty acid concentrations may be due to changes in cell membranes (Galliard 1968, Thompson 1984). The onset of fruit ripening is characterized by cell differentiation and tight control of enzyme synthesis (Brady 1987). Metabolic processes generate fatty acid peroxides which degrade polyunsaturated fatty acids (Brennen and Fraenkel 1977). In parallel to increases in oxidative stress, the activity of free-radical scavenging enzymes declines (Kalra and Brooks 1973, Rogiers et al. 1998, Wang and Jiao 2001). For some species, the relationship between concentrations of polyunsaturated fatty acids and fruit age was curvilinear. In the latter stages of fruit maturation, enzymes are induced to counter oxidation and membrane disorganization (Izzo et al. 1995, Rogiers et al. 1998). This may explain the increase in essential fatty acids. The mature fruits of some *Physalis* species are pigmented yellow, orange, red, or purple in color whereas the mature fruits of some other species are green. Pigment compounds such as flavonoids, anthocyanins, and carotenoids are antioxidants that can counter lipid peroxidation observed in mature fruits.

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Table 1. List of plant species examined in the study, the origin of the seeds, planting dates, and the ages of the plants and fruits at the time of harvest.

Plant species	Seed source	Planting date(s)	Plant age (days)	Fruit ages (days)
<u>Physalis species:</u>				
<i>P. ampla</i>	MEXICO, Jalisco, Municipality of Zapopan, Centro Universitario de Ciencias Agricolas y Agropecuarias of the Universidad de Guadalajara, growing as a weed in marginal areas around the university. Maintained for one generation in a common garden at the Centro Universitario de Ciencias Biologicas y Agropecuarias de la Universidad de Guadalajara, 2003; second generation in a common garden at the Estación de Biología Chamela UNAM.	1/13/2005	71-87	2-22
<i>P. angulata</i> (Mexico)	MEXICO, Jalisco, Municipality of Lagos de Moreno, Highway from Lagos de Moreno to Ojuelos de Jalisco, 6 km before the reservoir "Presa de Cuarenta," growing underneath Acacias; 21.46344N - 101.7988W, 1948 m. 6/IX/2004. Collected by M Bateman & G Gonzalez Lopez.	1/13/2005	63-78	4-24
<i>P. angulata</i> (North Carolina)	USA, NC, Goldsboro. Nicole Benda.	.	.	4-30
<i>P. cordata</i>	MEXICO, Jalisco, Municipality of La Huerta, Chamela, Estación de Biología Chamela UNAM, growing in a clearing by the greenhouses; 19.51333N, -105.0375 W. 30/X/2004. Collected by M Bateman & G Gonzalez Lopez.	1/25/2005	69-87	7-28
<i>P. minuta</i>	MEXICO, Colima, Manzanillo; 104°14'21.3"W, 18°59'38.1"N; growing on coastal dunes. Maintained for one generation in a common garden at the Centro Universitario de Ciencias Biologicas y Agropecuarias de la Universidad de Guadalajara, 2003; second generation in a common garden at the Estación de Biología Chamela UNAM. 16/XI/2004.	2/22/2005	64-71	2-21
<i>P. peruviana</i>	France, Paguigan, B & T World Seeds	3/9/2005	93-102	8-27
<i>P. philadelphica</i>	MEXICO, Jalisco, Municipality of La Huerta, town of Arroyo Seco, tomatillo field near beach; 19.3324 N, -104.9552 W, 10 m. 23/XI/2004. Collected by M Bateman & G Gonzalez Lopez. Seeds were collected and grown in a greenhouse: GERMANY, Thuringia, Jena, Max	1/13/2005	72-77	10-22
<i>P. pruinosa</i>	MEXICO, Jalisco, Compostela. Maintained for one generation in a common garden at the Centro Universitario de Ciencias Biologicas y Agropecuarias de la Universidad de Guadalajara, 2003; second generation in a common garden at the Estación de Biología Chamela UNAM.	1/13/2005	73-87	6-25
<i>P. pubescens</i>	MEXICO, Jalisco, Municipality of Ixtlahuacan del Rio, Dirt Road between the villages of Jagueycita and Coyotillo; growing under oak trees, alongside fields of corn and tomatillos; 20.948 N, -103.224722 W, 1881 m. 17/X/2004. Collected by M Bateman & G Gonz	2/18/2005	58-66	4-24
<i>P. solanaceous</i>	MEXICO, Jalisco, Municipality of Lagos de Moreno, Highway from Lagos de Moreno to Ojuelos de Jalisco, 6 km before the reservoir "Presa de Cuarenta", growing underneath Acacias; 21.46344N - 101.7988W, 1948 m. 6/IX/2004. Collected by M Bateman & G Gonzalez Lopez. S	2/18, 2/22/2005	75-109	13-31
<u>Other Solanaceae:</u>				
<i>Nicandra physalodes</i>	USDA, Agricultural Research Service; Southern Regional PI Station, Accession 10112	3/9/2005	79-90	3-26
<i>Solanum hirsutum</i>	George Kennedy	2/22/2005	160-171	

Table 2. Results of analysis of variance on the diameter, arcsine-square root transformed percent water content, and the natural log of the concentrations of linoleic and linolenic acids for fruits of nine *Physalis* species.

Effect	Fruit diameter			Percent water			Linoleic			Linolenic		
	df	F	P	df	F	P	df	F	P	df	F	P
<i>Physalis</i> species	9	4.24	0.0002	9	5.01	<0.0001	9	2.36	0.02	9	2.69	0.01
Log-fruit age	1	20.1	<0.0001	1	11.73	0.0007	1	6.14	0.01	1	8.53	0.004
Log-fruit age x <i>Physalis</i> species	9	4.94	<0.0001	9	4.54	<0.0001	9	2.24	0.02	9	2.72	0.006
Log-fruit age x Log-fruit age	1	7.41	0.0006	1	15.11	0.0001	1	6.53	0.01	1	6.79	0.01
Log-fruit age x Log-fruit age x <i>Physalis</i> species	9	4.15	<0.0001	9	3.44	0.0004	9	2.07	0.04	9	2.86	0.004

Table 3. Comparison fruit diameter (mm), percent water content, linolenic acid (mg/g dry weight), and linoleic acid (mg/g dry weight) of 10- and 25-day old fruits. Data presented are least square means \pm standard error. Values with the same letter do not differ significantly at $P < 0.05$.

Species	Fruit diameter		Water Content		Linolenic acid		Linoleic acid	
	10 days	25 days	10 days	25 days	10 days	25 days	10 days	25 days
<i>Physalis</i>								
<i>P. ampla</i>	4.12 c	8.69 cd	90.77 a	78.61 bc	3.68 ab	1.24 bc	14.38	17.50 ab
<i>P. angulata</i>								
Mexico	5.95 b	12.86 b	87.27 ab	76.95 c	3.01 ab	1.46 bc	10.82	20.95 ab
North Carolina	7.80 b	11.8 bc	85.49 abc	81.02 ab	1.27 b	0.40 c	9.13	7.78 b
<i>P. cordata</i>	6.22 b	8.08 d	90.06 a	82.56 bc	4.82 ab	1.54 bc	15.59	15.83 ab
<i>P. minuta</i>	3.72 c	8.35 d	88.19 ab	81.02 abc	3.29 ab	3.12 abc	13.79	53.58 ab
<i>P. peruviana</i>	5.18 bc	14.15 b	89.45 ab	78.61 bc	3.36 ab	1.82 bc	5.92	28.54 ab
<i>P. philadelphica</i>	3.79 c	23.51 a	88.19 ab	90.06 ab	4.82 ab	2.28 abc	20.25	12.29 ab
<i>P. pruinosa</i>	5.29 b	9.67 d	88.19 ab	79.43 bc	6.00 a	1.62 bc	12.44	8.56 b
<i>P. pubescens</i>	5.18 bc	5.38 e	90.65 a	92.84 a	2.81 ab	12.08 a	13.57	60.62 ab
<i>P. solanaceous</i>	2.32 c	3.00 f	74.37 bc	75.24 c	15.21 ab	3.18 ab	68.84	82.28 a
Other Solanaceae								
<i>Nicandra physalodes</i>	9.87 a	12.77 b	77.23 c	57.53 d	2.3 b	0.51 bc	16.18	11.63 b
	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe
<i>Solanum lycopersicum</i>	42.29	55.36	92.19	93.12	1.65	0.95	6.43	7.25

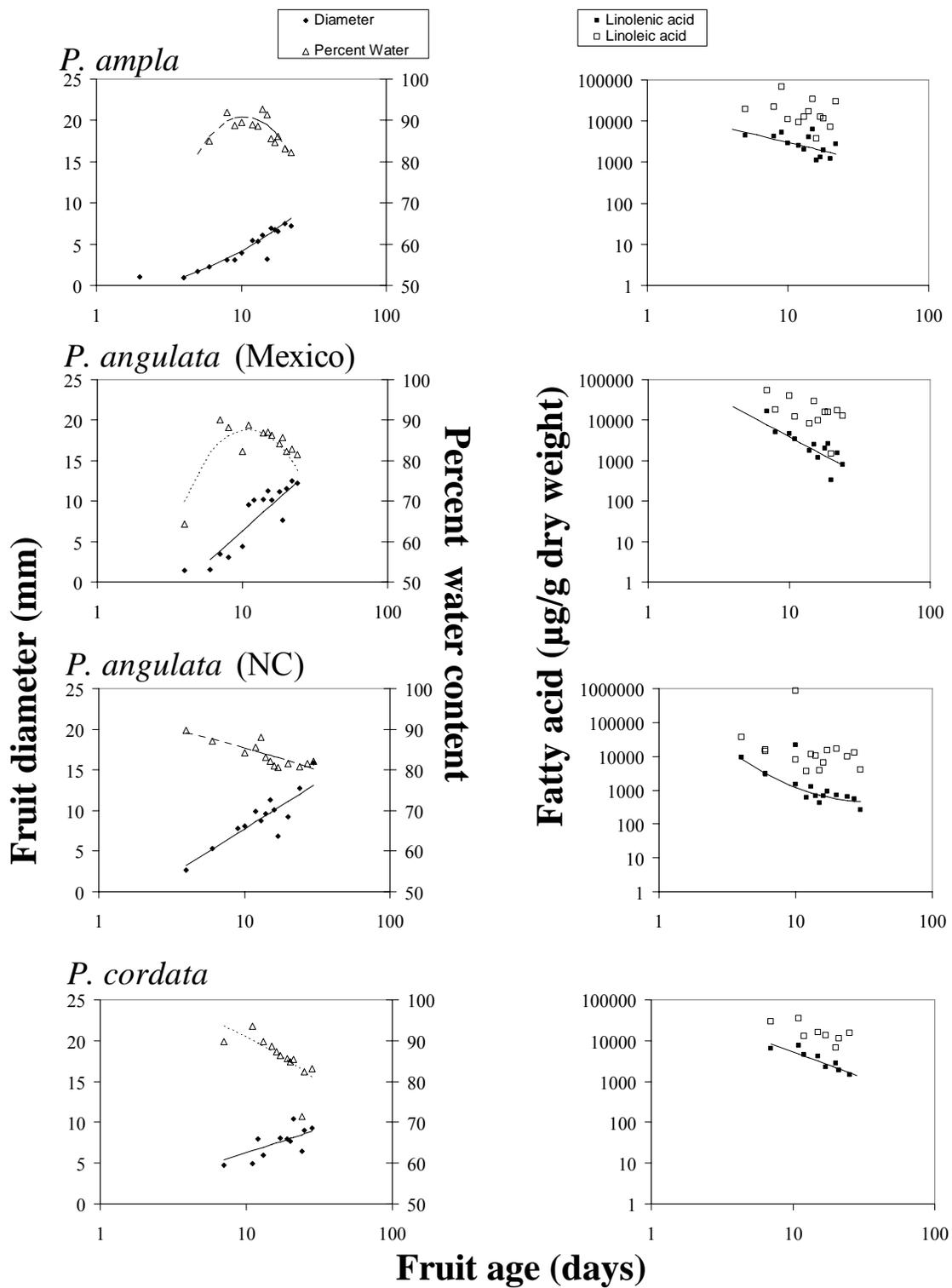


Fig. 1. Relationships between fruit age and measurements of fruit diameter, water content, and the concentrations of essential fatty acids for nine *Physalis* species, *Nicandra physalodes*, and tomato.

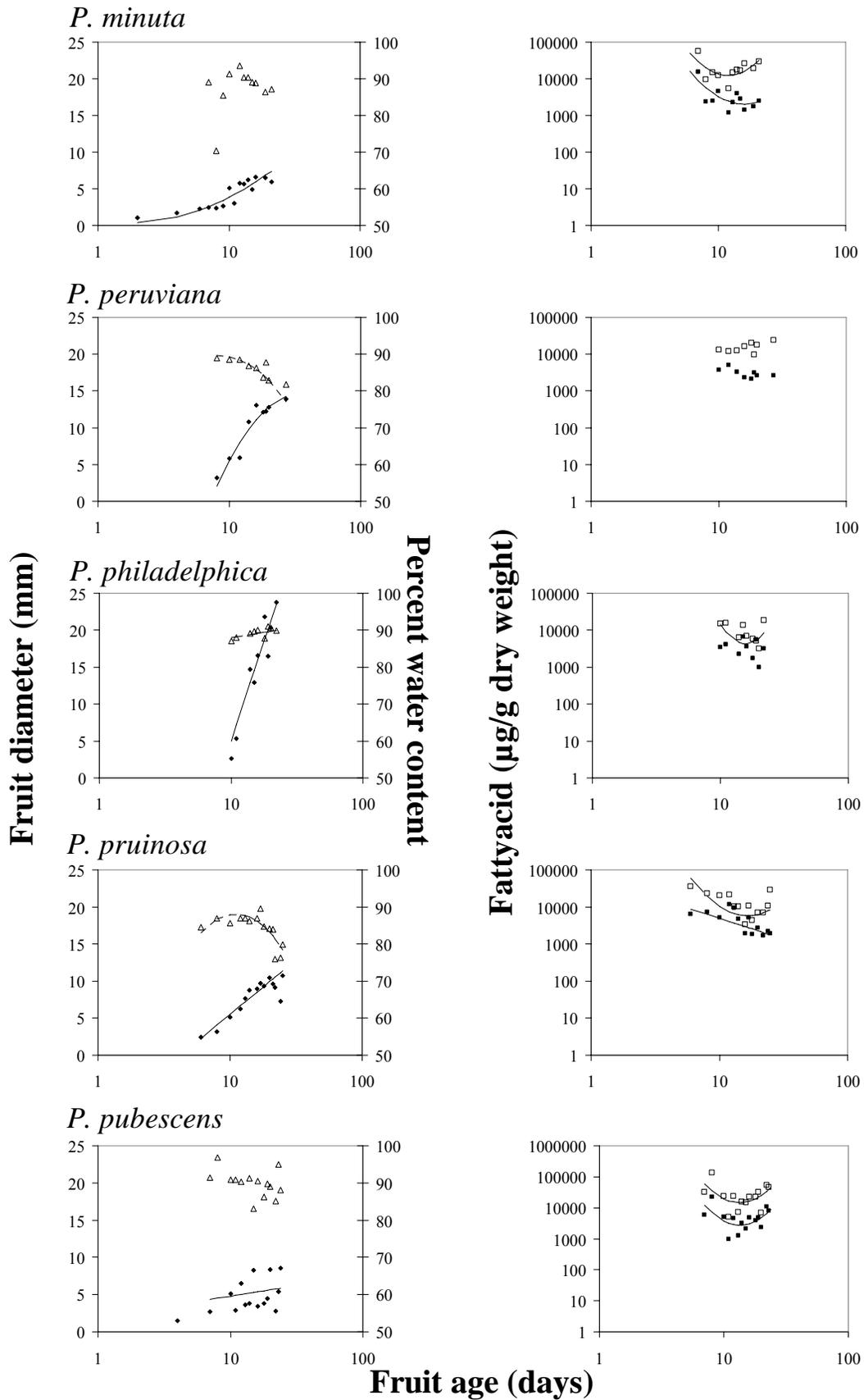


Fig. 1. (Continued)

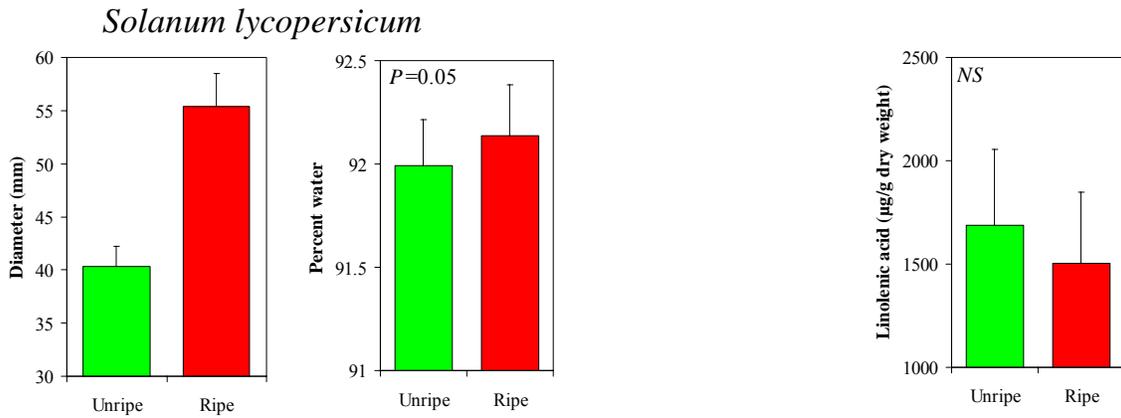
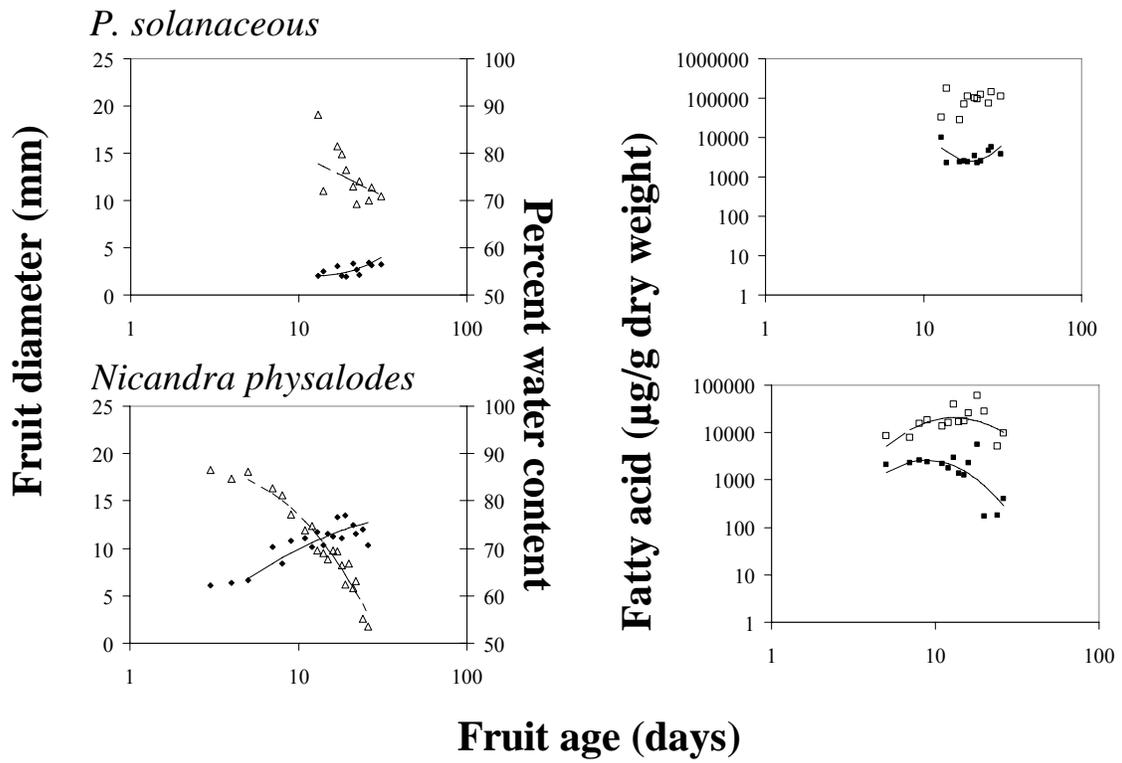


Fig. 1. (Continued)

Fig. 2. Comparison of immature and mature tomato fruits with respect to fruit diameter, water content, and the concentrations of the linolenic and linoleic fatty acids.

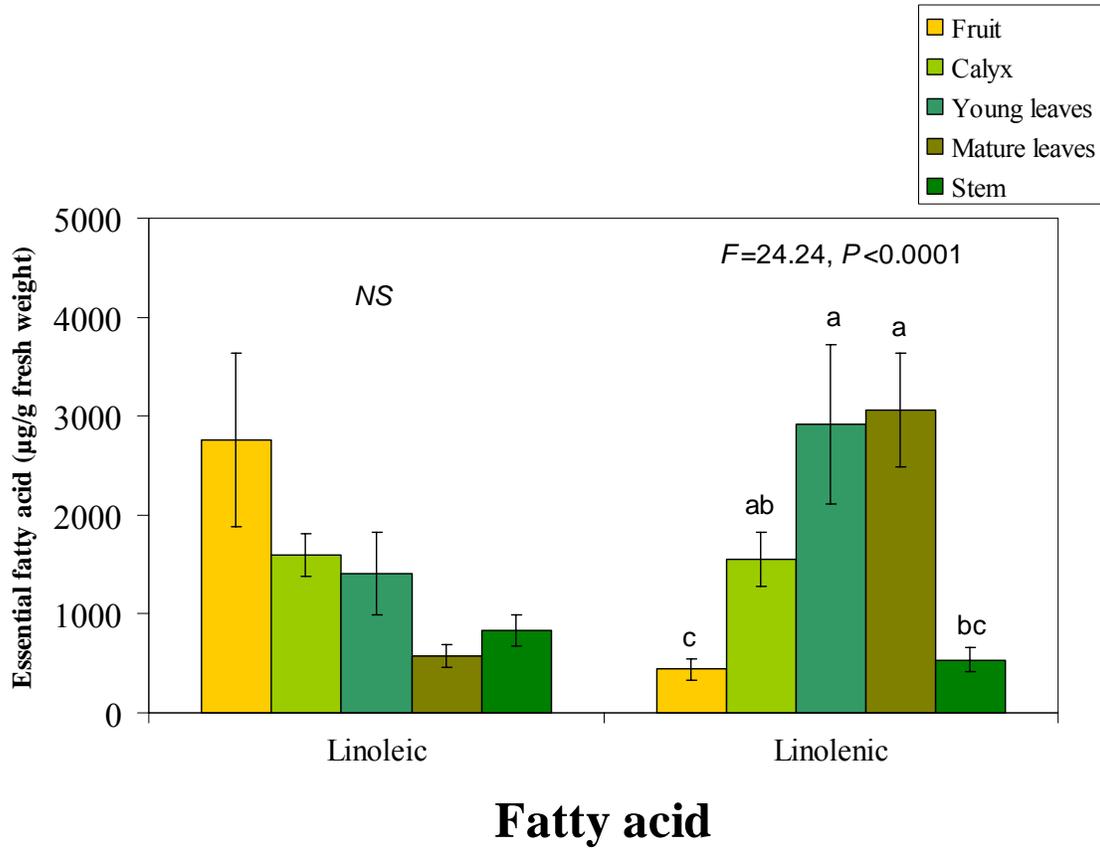


Fig. 3. Comparison of concentrations of linoleic and linolenic fatty acids in different tissues of *P. angulata* plants (Mexican accession).

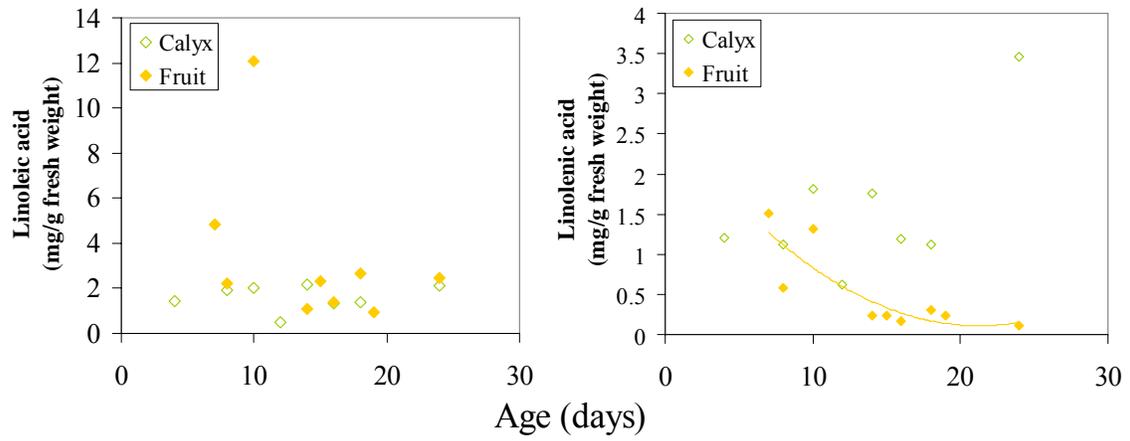


Fig. 4. Relationship between tissue age and the concentrations of essential fatty acids in the calyces and fruits of *P. angulata* plants (Mexican accession).

APPENDIX

Appendix A. Description of field sites in West-central Mexico, 2002 to 2004.

Location (Municipality, Town)	Longitude	Latitude	Elevation (m)	Data collection dates	Dominant vegetation	Disturbance	<i>Physalis</i> species	Germplasm
State of Aguascalientes:								
Los Caños	-102.45206	21.79325	1,700	9/7/2004	Subtropical scrub	Pasture	<i>P. angulata</i> , <i>P. nicandroides</i> , <i>P. solanaceus</i>	.
State of Colima:								
Km 141	-103.61959	19.3063897	841	8/31/2003	Subdeciduous tropical forest	Corn field	<i>P. cordata</i> , <i>P. lagascae</i> , <i>P. pruinosa</i>	X
Tecolapa	-103.8287	19.017701	160	9/29/2002, 8/11/2003	Tropical deciduous forest	Roadside	<i>P. lagascae</i> , <i>P. melanocystis</i>	X
San Antonio	-103.7178	19.4481148	1,157	8/11/2003	Subdeciduous tropical forest	Roadside	<i>P. pubescens</i>	X
Hierbabuena	-103.69284	19.4699366	1,401	8/11/2003	Subdeciduous tropical forest	Pasture	<i>P. ampla</i> , <i>P. pubescens</i>	.
Cuyutlan	-104.06667	18.9166667	54	8/11/2003	Mangroves	Roadside	<i>P. acutifolia</i> , <i>P. lagascae</i>	X
Cofradia Juarez	-103.96667	18.9176677	54	9/29/2002	Tropical deciduous forest	Roadside	<i>P. cordata</i>	.
Manzanillo, Playa Miramar	-104.49925	19.1335472	10	9/28/2002	Beach	Beach	<i>P. minuta</i>	.
Mazanillo, near Puente Tepelcates	-104.25265	19.0331498	13	8/11/2003, 8/31/2004, 9/21/2004, 10/15/2004	Mangrove	Corn field	<i>P. acutifolia</i> , <i>P. lagascae</i>	X
Manzanillo	-104.24022	18.9945112	29	9/29/2002, 8/31/2003, 10/4/2003, 8/31/2004, 9/21/2004, 10/15/2004	Dunes	Dunes	<i>P. minuta</i>	X
State of Jalisco:								
Zapopán, San Esteban	-103.35024	20.789039	1,370	8/5/2003	Tropical deciduous forest	Pasture	<i>P. ampla</i> , <i>P. angulata</i> , <i>P. pubescens</i>	X
Ixtlahuacán del Río, San José	-103.33333	20.8333333	1,100	10/22/2002	Tropical deciduous forest	Roadside	<i>P. ampla</i> , <i>P. angulata</i>	.
Ixtlahuacán del Río, Paso de Guadalupe	-103.35	20.8833333	900	10/22/2003	Tropical deciduous forest	Roadside	<i>P. hederifolia</i>	.
Ixtlahuacán del Río, San Miguel de Abajo	-103.27701	20.8222055	1,348	7/16/2003	Tropical deciduous forest	Tomatillo farm	<i>P. ampla</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i> , <i>P. pruinosa</i>	.
Ixtlahuacán del Río, Jagüey	-103.19907	20.9206707	1,856	9/4/2003	Conifer-oak forest	Tomatillo farm	<i>P. philadelphica</i> , <i>P. pubescens</i>	.
Ixtlahuacán del Río, Las Cruces	-103.11706	21.0005439	1,934	9/27/2003	Conifer-oak forest	Margin of field	<i>P. ampla</i> , <i>P. lagascae</i> , <i>P. philadelphica</i>	.
Ixtlahuacán del Río, Las Cruces	-103.11728	21.0142778	1,911	9/7/2004, 9/27/2004	Conifer-oak forest	Tomatillo farm	<i>P. philadelphica</i>	.
Ixtlahuacán del Río, Las Cruces	-103.11246	21.0189189	1,893	10/22/2002	Conifer-oak forest	Tomatillo farm	<i>P. philadelphica</i>	.
Ixtlahuacán del Río, Coyotillo	-103.10853	20.9458292	1,824	8/19/2003, 9/27/2003, 11/22/2003, 8/4/2004, 9/7/2004	Conifer-oak forest	Tomatillo farm	<i>P. ampla</i> , <i>P. angulata</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i> , <i>P. pubescens</i>	X
Ixtlahuacán del Río, Coyotillo	-103.12794	20.9492222	1,896	8/14/2004, 10/17/2004	Conifer-oak forest	Tomatillo farm	<i>P. ampla</i> , <i>P. philadelphica</i> , <i>P. pubescens</i>	.
Ixtlahuacán del Río, La Garruña	-103.14147	20.96525	1,903	8/14/2004	Conifer-oak forest	Tomatillo farm	<i>P. ampla</i> , <i>P. philadelphica</i> , <i>P. pubescens</i>	.
Ixtlahuacán del Río, La Majada	-103.13257	20.9530364	1,912	7/28/2003, 9/4/2003	Conifer-oak forest	Tomatillo farm	<i>P. philadelphica</i>	.
Ixtlahuacán del Río, Jagüeycito	-103.1664	20.975	1,960	10/22/2002, 9/4/2003	Conifer-oak forest	Tomatillo farm	<i>P. philadelphica</i> , <i>P. pubescens</i>	.
Acatic, Acatic	-102.91223	20.7445279	1,674	8/6/2003	Zacatal	Roadside	<i>P. chenopodiifolia</i>	X
Acatic, Acatic	-102.91086	20.8228061	1,685	8/6/2003	Zacatal	Pasture	<i>P. ampla</i> , <i>P. angulata</i> , <i>P. lagascae</i> , <i>P. nicandroides</i>	X

Appendix A. Description of field sites in West-central Mexico, 2002 to 2004.

Acatic, Acatic	-102.89768	20.8151985	1,645	8/6/2003	Conifer-oak forest	Pasture	<i>P. lagascae</i> , <i>P. waterfalli</i>	X
Lagos de Moreno, Presa de Cuarenta	-101.79987	21.4552386	1,931	8/6/2003, 8/7/2003, 11/1/2003, 8/15/2004, 9/6/2004,	Zacatal	Pasture	<i>P. angulata</i> , <i>P. cinerascens</i> , <i>P. hastatula</i> , <i>P. nicandroides</i> , <i>P. pubescens</i> , <i>P. solanaceous</i>	X
Lagos de Moreno, Rancho el Roble	-101.71869	21.5212222	1,973	10/16/2004 8/6/2003, 8/15/2004, 9/6/2004, 10/16/2004	Zacatal	Roadside	<i>P. angulata</i>	.
Ojuelos de Jalisco, Matanzas	-101.65701	21.6155322	2,216	8/7/2004	Zacatal	Pasture	<i>P. patula</i>	.
Ojuelos de Jalisco, Ojuelos de Jalisco	-101.56688	21.8759927	2,173	8/7/2003, 9/6/2004, 10/16/2004	Zacatal	Roadside	<i>P. patula</i>	.
Ojuelos de Jalisco, Chinampas	-101.81658	21.8160327	2,087	8/7/2003, 11/1/2003, 9/6/2004	Zacatal	Pasture	<i>P. glutinosa</i> , <i>P. nicandroides</i>	.
Zapopán, Los Colomos	-103.4	20.7166667	1,550	11/17/2002	Conifer-oak forest	Forest	<i>P. ampla</i> , <i>P. pubescens</i>	.
Zapopán, Centro Universitaria de Ciencias Agrícolas y Agropecuarias	-103.48333	20.78333	1,600	9/26/2002, 10/8/2002, 10/15/2002, 11/13/2002	Zacatal	Corn field	<i>P. ampla</i> , <i>P. angustiphysa</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i> , <i>P. pubescens</i>	X
Zapopán, Bosque la Primavera	-103.58333	20.65	1,660	9/27/2002, 10/17/2002	Conifer-oak forest	Roadside	<i>P. angulata</i> , <i>P. pubescens</i> , <i>P. nicandroides</i> , <i>P. pubescens</i>	X
Tala, Tala	-103.7	20.6666667	1,670	10/12/2002	Subtropical scrub	Corn field	<i>P. ampla</i> , <i>P. philadelphica</i> , <i>P. pubescens</i>	.
Tala, Tala	-103.7	20.6666667	1,670	10/12/2002	Subtropical scrub	Pasture	<i>P. ampla</i> , <i>P. nicandroides</i>	.
Tala, Las Navajas	-103.65184	20.5523023	1,474	7/29/2003	Subtropical scrub	Corn field	<i>P. ampla</i>	.
Tala, Bosque Escuela La Primavera	-103.63674	20.5979646	1,460	8/20/2003, 9/21/2003, 11/4/2003	Conifer-oak forest	Pasture	<i>P. ampla</i> , <i>P. nicandroides</i> , <i>P. pruinosa</i> , <i>P. pubescens</i>	X
Tala, Bosque Escuela La Primavera	-103.63824	20.5968135	1,472	8/20/2003, 9/21/2003, 11/4/2003	Conifer-oak forest	Corn field	<i>P. ampla</i> , <i>P. lagascae</i> , <i>P. nicandroides</i>	.
Etzatlan, Etzatlan	-104.08333	20.7666667	1,427	10/21/2002	Subtropical scrub	Corn field	<i>P. ampla</i> , <i>P. philadelphica</i>	.
Etzatlan, Oconahua	-104.16667	20.7166667	1,850	10/21/2002	Conifer-oak forest	Forest	<i>P. cordata</i> , <i>P. nicandroides</i> , <i>P. pruinosa</i>	.
San Marcos, San Marcos	-104.20622	20.79127	1,395	10/21/2002	Subtropical scrub	Corn field	<i>P. ampla</i> , <i>P. angulata</i> , <i>P. cordata</i> , <i>P. philadelphica</i>	.
San Marcos, San Marcos	-104.26333	20.7675	1,520	10/21/2002	Subtropical scrub	Roadside	<i>P. ampla</i> , <i>P. angulata</i> , <i>P. nicandroides</i>	.
Magdalena, before toll booth	-104.00978	20.9257758	1,342	8/9/2003	Subtropical scrub	Pasture	<i>P. ampla</i> , <i>P. angulata</i> , <i>P. nicandroides</i>	.
Cocula, Arroyo Colorado	-103.90833	20.30175	1,706	10/23/2002, 9/9/2004, 9/29/2004, 10/9/2004	Conifer-oak forest	Corn field	<i>P. ampla</i> , <i>P. angustiphysa</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i> , <i>P. pruinosa</i>	X
Juchitán, Los Corrales	-104.06667	20.0833333	1,200	10/23/2002	Tropical deciduous forest	Corn field	<i>P. hederifolia</i>	.
Tenamaxtlan, Tenamaxtlan	-104.14798	20.0929263	1,380	10/23/2002, 10/5/2003	Tropical deciduous forest	Corn field	<i>P. angulata</i> , <i>P. lagascae</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i>	X
Tenamaxtlan, Colotitlan	-104.16667	20.0833333	1,396	10/23/2002, 9/9/2004, 9/29/2004, 10/9/2004	Tropical deciduous forest	Corn field	<i>P. angulata</i> , <i>P. lagascae</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i>	.
Autlán de Navarro, Ahuacapan	-104.33333	19.6666667	1,179	10/24/2002	Tropical deciduous forest	Roadside	<i>P. cordata</i> , <i>P. philadelphica</i>	.
Autlán de Navarro, Chiquihuitan	-104.41718	19.7505289	1,008	10/5/2003	Tropical deciduous forest	Roadside	<i>P. lagascae</i> , <i>P. nicandroides</i> , <i>P. pruinosa</i> , <i>P. pubescens</i>	.
Autlán de Navarro, Jalocote	-104.42561	19.8247915	1,313	7/27/2003	Tropical deciduous forest	Roadside	<i>P. nicandroides</i>	X
Autlán de Navarro, Puerto Mazos	-104.38365	19.6961128	1,292	10/24/2002, 10/5/2003, 8/9/2004, 8/30/2004	Tropical deciduous forest	Roadside	<i>P. angulata</i> , <i>P. cordata</i> , <i>P. lagascae</i> , <i>P. microcarpa</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i>	X
Autlán de Navarro, Sierra de Manantlan	-104	19	1143	9/20/2004	Conifer-oak forest	Roadside	<i>P. pubescens</i>	.
Villa Purificación, Villa Purificación	-104.601	19.7166667	440	10/14/2002	Subdeciduous tropical forest	Roadside	<i>P. cordata</i> , <i>P. philadelphica</i>	.

Appendix A. Description of field sites in West-central Mexico, 2002 to 2004.

Location	Coordinates	Area (ha)	Year	Biome	Site Type	Species	Notes
Tonaya, La Rusia	-104.02953 19.7841389	849	8/9/2004	Tropical deciduous forest	Roadside	<i>P. lagascae</i>	
Tonaya, La Piña	-103.92794 19.7395556	890	8/9/2004, 8/30/2004, 9/20/2004, 10/10/2004	Conifer-oak forest	Tomatillo farm	<i>P. lagascae</i> , <i>P. nicandroides</i> , <i>P. pruinosa</i> , <i>P. philadelphica</i>	
San Gabriel, Izote	-105.27056 21.6305278	1,554	8/30/2004, 9/20/2004, 10/10/2004	Conifer-oak forest	Pasture	<i>P. orizabe</i> , <i>P. philadelphica</i>	
Ciudad Guzmán, Puerto Floripondio	-103.62053 19.6453056	2,397	10/24/2002, 8/12/2003, 8/30/2004, 9/20/2004, 10/10/2004	Conifer-oak forest	Logging road	<i>P. lignescens</i> , <i>P. orizabe</i>	
Ciudad Guzmán, Los Depositos	-103.531 19.6598333	1,699	8/30/2004, 9/20/2004, 10/10/2004	Conifer-oak forest	Tomatillo farm	<i>P. ampla</i> , <i>P. philadelphica</i> , <i>P. pubescens</i>	
Tlaquepaque, Cerro de Cuatro	-103.36316 20.6017518	1,806	8/5/2003	Conifer-oak forest	Corn field	<i>P. ampla</i> , <i>P. microcarpa</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i> , <i>P. pubescens</i>	
Zapotitan, Barranca de Huariche	-103.50657 20.3152152	1,683	8/16/2003, 9/3/2003, 9/17/2003, 10/12/2003	Conifer-oak forest	Corn field	<i>P. ampla</i> , <i>P. angulata</i> , <i>P. lagascae</i> , <i>P. microcarpa</i> , <i>P. nicandroides</i> , <i>P. pruinosa</i>	X
Jocotepec, Zapotitan	-103.46283 20.3331165	1,675	10/3/2002, 11/14/2002	Conifer-oak forest	Corn field	<i>P. sulfurea</i> , <i>P. ampla</i> , <i>P. angulata</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i> , <i>P. pruinosa</i>	X
Jocotepec, Potrero el Llano	-103.35097 20.345946	1,756	9/3/2003, 9/17/2003, 10/12/2003	Conifer-oak forest	Corn field	<i>P. ampla</i> , <i>P. angulata</i> , <i>P. lagascae</i> , <i>P. philadelphica</i> , <i>P. pubescens</i>	X
Sayula, Sayula	-103.51974 19.8788789	1,364	8/31/2003	Subtropical scrub	Roadside	<i>P. nicandroides</i>	X
Tecalitlán, Sierra del Halo	-103.30389 19.3847222	2,010	10/24/2002	Conifer-oak forest	Logging road	<i>P. philadelphica</i> , <i>P. subrepens</i>	
Tonila, in route to La Esperanza	-103.51667 19.4333333	1500	9/29/2002	Tropical, subdeciduous forest	Margin of corn field	<i>P. nicandroides</i> , <i>P. philadelphica</i>	
Puerto Vallarta, Mismaloya	-105.26678 20.5173657	70	2X	Palmar	Forest	unknown <i>Physalis</i> spp	
La Huerta, La Fortuna	-105.108 19.6012	10	10/14/2004, 10/27/2004, 11/8/2004, 11/11/2004, 11/14/2004, 11/17/2004	Tropical deciduous forest	Tomatillo farm	<i>P. philadelphica</i>	
La Huerta, San Mateo	-105.1005 19.5888	10	11/11/2004, 11/14/2004, 11/17/2004, 11/20/2004, 11/23/2004, 11/26/2004, 11/29/2004	Tropical deciduous forest	Tomatillo farm	<i>P. philadelphica</i>	
La Huerta, Xametla	-105.08508 19.53725	2.5	8/11/2004, 9/2/2004, 9/24/2004, 10/14/2004	Beach	Beach	<i>P. minuta</i>	
La Huerta, Chamela	-105.04925 19.5241944	35	7/12/2003, 7/26/2003, 8/11/2004, 9/2/2004, 9/23/2004, 10/14/2004	Tropical deciduous forest	Riverbed	<i>P. cordata</i> , <i>P. leptophylla</i> , <i>P. philadelphica</i> , <i>P. pruinosa</i> , <i>P. pubescens</i>	
La Huerta, Estación de Biología Chamela	-105.03694 19.5108611	70	10/14/2004, 9/3/2004, 9/22/2004, 9/24/2004, 10/6/2004	Tropical deciduous forest	Forest	<i>P. leptophylla</i> , <i>P. pruinosa</i>	
La Huerta, Estación de Biología Chamela	-105.04342 19.4986944	70	9/3/2004, 9/22/2004, 10/6/2004	Tropical deciduous forest	Roadside	<i>P. cordata</i>	
La Huerta, Careyitos	-105.02606 19.4376389	10	8/11/2004, 9/2/2004, 9/24/2004, 10/13/2004	Beach	Beach	<i>P. minuta</i>	
La Huerta, Villa San Francisco	-104.9708 19.388	15	10/5/2004, 11/11/2004, 11/14/2004, 11/17/2004, 11/21/2004, 11/24/2004, 11/26/2004, 11/29/2004	Tropical deciduous forest	Tomatillo farm	<i>P. philadelphica</i>	
La Huerta, Arroyo Seco	-104.9552 19.3324	10	10/13/2004, 10/27/2004, 11/5/2004, 11/8/2004, 11/11/2004, 11/14/2004, 11/17/2004	Tropical deciduous forest	Tomatillo farm	<i>P. philadelphica</i>	
La Huerta, Arroyo Seco	-104.9552 19.3324	10	10/13/2004	Beach	Beach	<i>P. minuta</i>	
Cihuatlan, El Central	-104.44701 19.1462129	71	8/10/2003	Palmar	Roadside	<i>P. cordata</i>	
State of Michoacán:							
El Faro	-104.25285 19.0333834	17	8/30/2003, 10/4/2003	Beach	Beach	<i>P. cordata</i> , <i>P. minuta</i>	
Playa el Tunel	-103.0665 18.1937437	26	8/30/2003	Beach	Beach	<i>P. cordata</i>	
Bahía Maruata	-103.50916 18.3490491	2	8/30/2003, 10/4/2003	Beach	Beach	<i>P. cordata</i> , <i>P. minuta</i>	
Brasiles			10/29/2002		Near houses	<i>P. melanocystis</i>	

Appendix A. Description of field sites in West-central Mexico, 2002 to 2004.

Canuto Neri, Acapetlahuaya	-100.06667	18.4166667	1364	10/29/2002	.	Field	<i>P. cordata</i> , <i>P. philadelphica</i>	.	
Canuto Neri, Acapetlahuaya	-100.06667	18.4166667	1364	10/29/2002	.	Field	<i>P. pruinosa</i>	.	
Cabeza Lazaro Crdenas	.	.	.	10/29/2002	.	Roadside	<i>P. melanocystis</i>	.	
Benito Juárez, El Bosque	-100.475	19.24307	1,240	10/28/2002	.	Tomatillo farm	<i>P. ampla</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i> , <i>P. pruinosa</i>	.	
Zitácuaro, El Gigante	-100.39667	19.3230556	1,710	10/28/2002	.	Roadside	<i>P. angulata</i> , <i>P. nicandroides</i> , <i>P. pubescens</i>	.	
Las Tuzantlas, Las Juntlas	-100.64167	19.19345	690	10/28/2002	.	Corn field	<i>P. ampla</i> , <i>P. philadelphica</i> , <i>P. pruinosa</i>	.	
Puente Tuzantlas	-100.56667	19.2	594	10/28/2002	.	Roadside	<i>P. pruinosa</i>	.	
State of Nayarit:									
Compostela	-104.80839	21.2171171	1,100	8/9/2003	Tropical deciduous forest	Roadside	<i>P. pruinosa</i>	X	
Las Varas, San Francisco	-105.41498	20.9017518	12	8/9/2003,	Beach	Beach	<i>P. cordata</i> , <i>P. minuta</i>	.	
Punta Mita	-105.52028	20.775	10	11/8/2003	Beach	Beach	<i>P. cordata</i> , <i>P. minuta</i>	.	
Rancho Mezcales	-105.29511	20.7233901	10	8/10/2003	Mangroves	Along trail	<i>P. acutifolia</i>	.	
State of Zacatecas:									
Salitre	-102.88747	22.1599099	2,415	9/16/2003	Subtropical scrub	Roadside	<i>P. angulata</i> , <i>P. nicandroides</i> , <i>P. philadelphica</i> , <i>P. solanaceous</i>	X	
Near Presa	-102.82816	22.1314648	1,888	9/13/2003	Subtropical scrub	Roadside	<i>P. angulata</i> , <i>P. lagascae</i> , <i>P. solanaceous</i>	.	
La Quemada	-102.83433	23.2666667	1,971	11/10/2002,	Zacatal	Roadside	<i>P. hederifolia</i>	.	
Tropico de Cancer	-103.05566	23.431615	2,128	9/13/2003	Zacatal	Pasture	<i>P. hederifolia</i> , <i>P. nicandroides</i>	.	

Appendix B. Insects found in association with *Physalis* plants in the field and in the common gardens in West-Central Mexico. Species determinations: 1. Ricardo Ayala, UNAM. 2. Felipe Noguera, UNAM. 3. David Furth, NMNH. 4. Melanie Bateman, NCSU. 5. Alma Solis, Research Entomologist, SEL (Systematic Entomology Laboratory). 6. Natalia J. Vandenberg, Entomologist, SEL

Hymenopteran pollinators¹:

Generalists:

Apis mellifera (L.)
Trigona (Friesemoleitta) nigra Cresson
Scaptotrigona hellwegeri Friese
Tetraloniella spp.
Exomalopsis spp.

Solanaceae specialists:

Trigona fulviventris Guerin
Perdita spp.

Folivores:

Parorectis rugosa (Boh) (Chrysomelidae)²
Leptinotarsa haldemani (Rogers) (Coleoptera: Chrysomelidae)²
Epitrix spp. (Coleoptera: Chrysomelidae)³
Halticus bractatus (Say) (Hemiptera: Miridae)³
As-yet-unidentified Criocerinae (Coleoptera)

Frugivores:

Heliothis subflexa Guenée (Lepidoptera: Noctuidae)⁴
Lineodes fontella Walsingham (Lepidoptera: Crambidae)⁵

Stem borers:

Languria spp. (Coleoptera: Erotylidae)⁶
As-yet-unidentified Curculionid beetle

Appendix C. SAS codes for some key statistical tests.

From Chapter 2 – Logistic regression on *H. subflexa* infestation to *Physalis* species over time and at multiple sites in West-Central Mexico.

```
proc genmod data=m; class plant location;
    model infected /totplants = location plant date plant*date
/type3 link=logit pscale;
run;
```

From Chapter 5 - Analysis of variance on fruit diameter (diameter), the arcsine-square root transformation of percent water content (apctwater), the natural log transformation of linolenic acid concentration (lolenenic), and the natural log transformation of linoleic acid concentration (linoletic):

```
proc glm data=together;
    where species in
    ('ampla', 'angulata', 'cordata', 'minuta', 'peruviana', 'philadelphica',
    ', 'pruinosa', 'pubescens', 'solanaceous', 'angulatanc'
    /*, 'nicandra'*/);
    class id species;
    model diameter apctwater llinolenic llinoleic = species logage
    species*logage logage*logage species*logage*logage /htype=1
    htype=3;
    random id(species);
run;
```

Alternative model for analyses of percent infestation to field plants:

```
proc mixed data=m;
    class plant location;
    model propinf = plant date plant*date;
    random location plant*location;
    lsmeans plant / at date=270 pdiff; run;
```