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Research

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Use of Chemical and Nonchemical Methods for the Control of *Varroa destructor* (Acari: Varroidae) and Associated Winter Colony Losses in U.S. Beekeeping Operations

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Abstract

The parasitic mite *Varroa destructor* (Acari: Varroidae) is a major cause of overwintering honey bee (*Apis mellifera*) colony losses in the United States, suggesting that beekeepers must control *Varroa* populations to maintain viable colonies. Beekeepers have access to several chemical varroacides and nonchemical practices to control *Varroa* populations. However, no studies have examined large-scale patterns in *Varroa* control methods in the United States. Here we used responses from 4 yr of annual surveys of beekeepers representing all regions and operation sizes across the United States to investigate use of *Varroa* control methods and winter colony losses associated with use of different methods. We focused on seven varroacide products (amitraz, coumaphos, fluvalinate, hop oil, oxalic acid, formic acid, and thymol) and six nonchemical practices (drone brood removal, small-cell comb, screened bottom boards, powdered sugar, mite-resistant bees, and splitting colonies) suggested to aid in *Varroa* control. We found that nearly all large-scale beekeepers used at least one varroacide, whereas small-scale beekeepers were more likely to use only nonchemical practices or not use any *Varroa* control. Use of varroacides was consistently associated with the lowest winter losses, with amitraz being associated with lower losses than any other varroacide product. Among nonchemical practices, splitting colonies was associated with the lowest winter losses, although losses associated with sole use of nonchemical practices were high overall. Our results suggest potential control methods that are effective or preferred by beekeepers and should therefore inform experiments that directly test the efficacy of different control methods. This will allow beekeepers to incorporate *Varroa* control methods into management plans that improve the overwintering success of their colonies.

Key words: apiculture, colony loss, varroacide, *Varroa* control

The parasitic mite *Varroa destructor* (Acari: Varroidae) (Anderson and Trueman 2000) plays a key role in the mortality of overwintering honey bee (*Apis mellifera* L.) colonies (Boecking and Genersch 2008, Le Conte et al. 2010, Rosenkranz et al. 2010, van Dooremalen et al. 2012, Döke et al. 2015). *Varroa destructor* weakens honey bee colonies by reducing immune response (Gregory et al. 2005, Yang and Cox-Foster 2005), vectoring viruses (Boecking and Genersch 2008), and impairing physiological development (Amdam et al. 2004). The adverse effects of *Varroa* may be amplified by interactions between *Varroa* and other colony stressors such as pesticides or reduced nutrition (Rosenkranz et al. 2010, van Dooremalen et al. 2013). Thus, maintaining healthy honey bee colonies in the United States is dependent on control of *Varroa* populations. U.S. beekeepers have access to a number of synthetic and natural varroacides as well as nonchemical practices to help manage *Varroa* populations in their operations. To date, however, few studies have examined beekeeper *Varroa* control practices or associated these practices with operational success (Giacobino et al. 2016).

V. destructor is native to Asia, but its range expanded to Europe in the 1970s. It was first found in the United States in the 1980s (Oldroyd 1999, Rosenkranz et al. 2010). Its original host, *Apis cerana*, limits *V. destructor* populations by allowing *V. destructor* to reproduce only in drone brood and through hygienic behaviors such as self-grooming and uncapping and removing parasitized brood (Oldroyd 1999, Rath 1999, Rosenkranz et al. 2010). Unlike in *A. cerana*, *V. destructor* is able to reproduce in worker cells of

A. mellifera, which increases the mite population exponentially, eventually killing the colony (Oldroyd 1999).

A number of synthetic chemicals are available in the United States for *Varroa* control. The most common synthetic varroacides in the United States are the formamidine amitraz, the organophosphate coumaphos, and the pyrethroid fluvalinate. These varroacides are easy to apply and relatively inexpensive. They are also lipophilic, and so they do not easily contaminate honey (Rosenkranz et al. 2010). However, these compounds accumulate in wax, thereby compromising colony health (Johnson et al. 2009, Traynor et al. 2016). Moreover, the efficacy of many of these products in the United States has become limited, as *Varroa* populations have developed resistance to all of them (Milani 1999, Pettis 2004, Sammataro et al. 2005, Johnson et al. 2010). Thus, other methods are needed to keep *Varroa* populations under control.

As an alternative to synthetic varroacides, several natural varroacides are used for *Varroa* control. These include the organic acids hop oil, formic acid, and oxalic acid, and the essential oil thymol. Because these compounds are either hydrophilic or volatile, they are unlikely to accumulate in comb wax. In addition, mites are unlikely to develop resistance to them (Milani 1999, Rosenkranz et al. 2010). However, the effects of these natural varroacides are often dependent on colony and hive conditions and environmental factors, and thus, their efficacy is more variable than that of synthetic varroacides (Rosenkranz et al. 2010).

Varroa resistance to synthetic varroacides and the variable efficacy of natural varroacides highlight the need to incorporate nonchemical management strategies to help control *Varroa* populations (Ruffinengo et al. 2014). Several nonchemical *Varroa* control approaches, including drone brood removal (Calderone 2005), screened bottom boards (Harbo and Harris 2004, Delaplane et al. 2005), and splitting colonies (Evans 2015, Milbrath 2017, Cornell University College of Agriculture and Life Sciences 2018), do slow mite population growth. The magnitude of the reduced population growth, however, is often insufficient to justify the labor expense on a large scale (Sammataro et al. 2000), and these approaches do not preclude the need for some form of chemical intervention later in the season (e.g., Delaplane et al. 2005, Wantuch and Tarpy 2009). Other nonchemical approaches, such as dusting with powdered sugar, have shown promise in laboratory assays but not under field conditions (Fakhimzadeh 2001, Aliano and Ellis 2005, Rosenkranz et al. 2010). Another approach, small-cell comb, has been hypothesized to impede mite production by reducing the amount of space between bee pupae and cell walls (Martin and Kryger 2002) or by reducing the development time of honey bee pupae so that adult bees emerge before offspring mites mature (Camazine 1986, Siuda and Wilde 1996, Siuda et al. 1996). However, use of small-cell comb has failed to show any measurable reduction in mite populations in empirical studies (Ellis et al. 2009a, Berry et al. 2010, Seeley and Griffin 2011).

Over the long term, the best solution for *Varroa* control is the use of bees that exhibit resistance or tolerance to *V. destructor*, as is the case for the mite's native host, *A. cerana* (Rath 1999). Several lines of mite-resistant or tolerant honey bees have been identified or developed through selection for resistance traits (Delaplane et al. 2005, Le Conte et al. 2007, Ward et al. 2008, Kirrane et al. 2018). A number of these resistant lines, including *Varroa* Sensitive Hygiene (VSH), Minnesota hygienic, Purdue hygienic, and Russian, are commercially available in the United States. However, the purity of these commercial strains is not well-defined, and this approach may only reduce or delay, rather than eliminate, the need for chemical intervention (Delaplane et al. 2005, Ward et al. 2008).

The current descriptive study examines patterns in use of varroacides and nonchemical management practices by U.S. beekeepers and the winter colony losses associated with these *Varroa* control methods. We describe reported patterns in *Varroa* control strategies employed among different beekeeper groups and compare operational winter loss rates among beekeepers who reported different mite management approaches. This is the first study to report patterns in chemical and nonchemical *Varroa* control methods used by beekeepers across the United States and provides insight into which methods for *Varroa* control in past years may be associated with the highest colony survivorship.

Methods

Loss and Management Survey

The data used for this study were derived from responses to questions in the annual Bee Informed Partnership Honey Bee Colony Loss and Management Surveys (e.g., Lee et al. 2015, Seitz et al. 2016, Kulhanek et al. 2017). Each survey covered the period from 1 April to 31 March, and the current study uses surveys conducted annually from 2013–2014 through 2016–2017. Participants were recruited using beekeeping organizations' distribution lists and using snowball sampling, in which participants are asked to assist researchers with recruiting other potential participants. Thus, respondents were not randomly chosen, and so the responding population should not be considered representative of the nation's beekeeper community. Although these survey participants may not be representative of the U.S. beekeeping population as a whole, their responses provide insight into what management practices beekeepers use and which practices are associated with increased colony survivorship.

The data reported in the current study come from respondents who answered questions regarding their use of chemical and nonchemical *Varroa* control treatments and practices. These respondents also provided information that permitted the calculation of their operational winter colony loss rate (Steinhauer et al. 2014). Some responses were illogical or incomplete, and these responses were filtered using R v. 3.4.1 (R Core Team 2017) and excluded from analyses. Individual operations may be represented more than once if respondents participated in the survey in more than 1 yr. However, to keep responses confidential, we gave each respondent a different ID each year, and we consider responses to be independent among years.

Categorizing Survey Responses

We divided survey respondents into four groups ("operation types"), as described by Steinhauer (2017), based on region and operation size. Small-scale beekeepers (those managing fewer than 50 colonies on 1 October, the start of winter) were divided into northern and southern regions based on the nine U.S. climate regions identified by the NOAA National Centers for Environmental Information (Karl and Koss 1984). We defined the northern region as the region comprising states from the northwest, west north central, east north central, central, and northeast climate regions, and we defined the southern region as the region comprising states from the west, southwest, south, and southeast climate regions. Large-scale beekeepers (those managing 50 or more colonies on 1 October) were divided into a group that kept colonies in more than one state over the course of a year and a group that kept all colonies in a single state throughout the year. Previous work has found that beekeepers in each group use different management practices (Steinhauer 2017), and so we stratify our results by these groupings as appropriate.

We examined survey question responses pertaining to the use of varroacides and nonchemical *Varroa* control methods (Table 1). We summarized the use of *Varroa* control methods by presenting the proportion of qualified respondents who used a given category, type, or specific product or practice. We also examined combinations of products or practices used over the course of the year and report the five most frequently used combinations of products and practices within each operation type.

To determine whether there were relationships between specific *Varroa* control practices and operational winter loss (defined as colonies lost from 1 October to 31 March), we assigned respondents into mutually exclusive groups based on the *Varroa* control methods they reported. Note that we do not know whether respondents obtained commercial varroacide products or whether they used homemade formulations, nor did we consider the number of applications or dosage used of individual products.

Statistical Analyses

Winter Loss

To determine whether differences in *Varroa* control methods were associated with differences in winter colony loss, we conducted separate generalized linear mixed models for each of the following fixed effects (see Table 1 for further details of fixed-effect groupings): 1) *Varroa* control category, 2) varroacide chemical type, 3) number of varroacide products reported, 4) individual varroacide products used as the only *Varroa* control method, 5) combinations of varroacides, 6) number of nonchemical management practices reported, 7) individual nonchemical practices used as the only *Varroa* control method, 8) mite-resistant status of genetic lines used in an operation, and 9) combinations of nonchemical *Varroa* control practices. We conducted all analyses using the '*glmer*' function in the *lme4* package in R. Because colony losses are presented as a proportion, each model assumed a binomial distribution and used a logit link function. For the analyses of individual varroacide products and individual nonchemical practices, we pooled all operation types due to insufficient numbers of respondents within single operation types, and we included both survey year and operation type as random effects. For all other fixed effects, we conducted a separate analysis for each operation type that included survey year as a random effect. Fixed-effect groupings with fewer than five respondents were excluded from analyses to maintain confidentiality of respondents.

Table 1. *Varroa* control methods included in this study, grouped by category and type within each category.

Varroa control methods		
Category	Type	Specific product or practice
Varroacide	Synthetic chemical	Amitraz
		Coumaphos
		Fluvalinate
	Organic acid	Hop oil
		Formic acid
		Oxalic acid
	Essential oil	Thymol
Nonchemical	Biotechnical	Drone brood removal
		Small-cell comb
	Physical	Screened bottom boards
		Powdered sugar
	Genetic	Mite-resistant lines
	Brood interruption	Splitting colonies
No control	None	None

When generalized linear models indicated a significant difference, we followed up with Tukey–Kramer multiple comparisons tests using the *multcomp* package in R and a Bonferroni-adjusted alpha level to determine which groups were associated with different winter losses.

We note that because we did not examine *Varroa* reduction in response to *Varroa* control practices, and we do not know whether colonies died as a result of *Varroa*, we cannot establish a direct or causal link between *Varroa* control practices and winter colony losses. However, *V. destructor* is thought to be a key driver of honey bee colony mortality (e.g., Rosenkranz et al. 2010). Thus, the *Varroa* control methods used by beekeepers in the current study are likely to have played a role in winter colony mortality.

Use Over Time

We assigned *Varroa* control category and varroacide chemical type into mutually exclusive groups, and thus, any changes over time in use of one category or type would be nonindependent from changes in use of other categories or types. Therefore, to determine whether the frequency of use of 1) different *Varroa* control categories and 2) different varroacide chemical types changed over time, we conducted multinomial logistic regression analyses for each operation type using the '*multinom*' function in the *nnet* package in R. *Varroa* control category and varroacide chemical type, respectively, were modeled as outcome variables, and for both analyses, survey year was modeled as the predictor variable. If a model was significant, we conducted a logistic regression analysis for each *Varroa* control category or varroacide chemical type, with use and nonuse as the possible outcomes, using the '*glm*' function in R and a Bonferroni-adjusted alpha level. We conducted separate tests for each operation type.

Rather than categorizing yearly reported use of nonchemical methods into mutually exclusive groups (which would have required us to examine all possible combinations of the six practices), we investigated reported use over time for each nonchemical practice independently. We conducted separate Cochran–Armitage tests for trend using the '*CochranArmitageTest*' function in the *DescTools* package in R. The response variable in each test was use of the management practice (used or did not use) and the explanatory variable was survey year (from 2013–2014 through 2016–2017). We analyzed each operation type separately. Because we conducted six tests within each operation type (one for each nonchemical management practice), we used a Bonferroni-adjusted alpha level of 0.0083.

Results

In total, 18,901 respondents provided sufficient valid responses to allow for the calculation of operational winter colony loss. On average, qualified respondents lost 23.0% (95% CI: 21.0–25.1%) of their colonies over winter. We refer to this average as the grand mean winter loss throughout the remainder of this article and use it as a baseline to compare across different subsets of the respondent pool.

Categories of *Varroa* Control Methods Used

Frequencies of Use and Associated Winter Losses

We obtained responses regarding *Varroa* control category from 11,989 northern small-scale; 6,071 southern small-scale; 322 multi-state large-scale; and 519 single-state large-scale beekeepers across the 2013–2014 through 2016–2017 survey years. Small-scale beekeepers most frequently reported using a combination of varroacides and nonchemical methods (northern: 55.5%; southern: 46.7% of respondents). The second most common category of mite control was nonchemical practices only (northern: 20.8%;

southern: 28.9%), followed by no known *Varroa* control (northern: 13.6%; southern: 18.5%), and lastly, varroacide(s) only (northern: 10.1%; southern: 5.8%). Most large-scale beekeepers reported using varroacides. Specifically, 65.2% of multi-state large-scale beekeepers reported using varroacides only and 32.0% reported a combination of varroacides and nonchemical practices, whereas 53.4% of single-state large-scale beekeepers reported using a combination of varroacides and nonchemical practices, and 30.8% reported using varroacides only. Few large-scale beekeepers reported using only nonchemical practices (multi-state: 2.2%; single-state: 9.4%), and even fewer reported using no known *Varroa* control (multi-state: 0.6%; single-state: 6.4%).

For all operation types, winter colony losses differed among respondents who reported using different *Varroa* control categories (northern small-scale: χ^2_3 = 1,331.5, $P < 0.0001$; southern small-scale: χ^2_3 = 122.3, $P < 0.0001$; multi-state large-scale: χ^2_2 = 891.0, $P < 0.0001$; single-state large-scale: χ^2_3 = 2,269.4, $P < 0.0001$). Both groups of small-scale beekeepers who reported using varroacides, either alone or in combination with nonchemical practices, experienced significantly lower winter losses than those who did not report using varroacides (Fig. 1a and b). Northern small-scale beekeepers who reported using only nonchemical management practices averaged 11.9% lower losses than those who used no *Varroa* control (Fig. 1a). Winter losses were not significantly different for southern small-scale beekeepers who reported using only nonchemical practices versus no known *Varroa* control (Fig. 1b). Tukey–Kramer tests indicated that for both multi-state and single-state large-scale beekeepers, winter losses were significantly different among the practitioners of all categories of *Varroa* control methods. Varroacide use was associated with the lowest winter losses (Fig. 1c and d). For multi-state beekeepers, reported use of the combination of varroacides and nonchemical methods was associated with the lowest winter losses (Fig. 1c), and for single-state beekeepers, the reported use of varroacides alone was associated with the lowest winter losses (Fig. 1d).

Reported Frequency of Use Over Time

Multinomial logistic regression analyses indicated that for both regions of small-scale beekeepers, reported use of different categories of *Varroa* control changed over time (northern: χ^2_3 = 42.5, $P < 0.0001$; southern: χ^2_3 = 18.6; P = 0.0003). Reported use of varroacides as the only category of *Varroa* control increased significantly for both of these groups (31.4% and 76.6% increase from 2013–2014 to 2016–2017 for northern and southern regions, respectively; Fig. 2a and b). Reported use of nonchemical control methods by northern small-scale beekeepers also changed significantly over time (Fig. 2a). Reported use of a combination of varroacides and nonchemical practices increased by 9.8%, reported use of nonchemical practices alone decreased by 21.5%, and use of no known *Varroa* control decreased by 27.2% from 2013–2014 to 2016–2017. Multi-state large-scale beekeepers did not exhibit any significant change over time in reported use of different categories of *Varroa* control (χ^2_3 = 0.31,

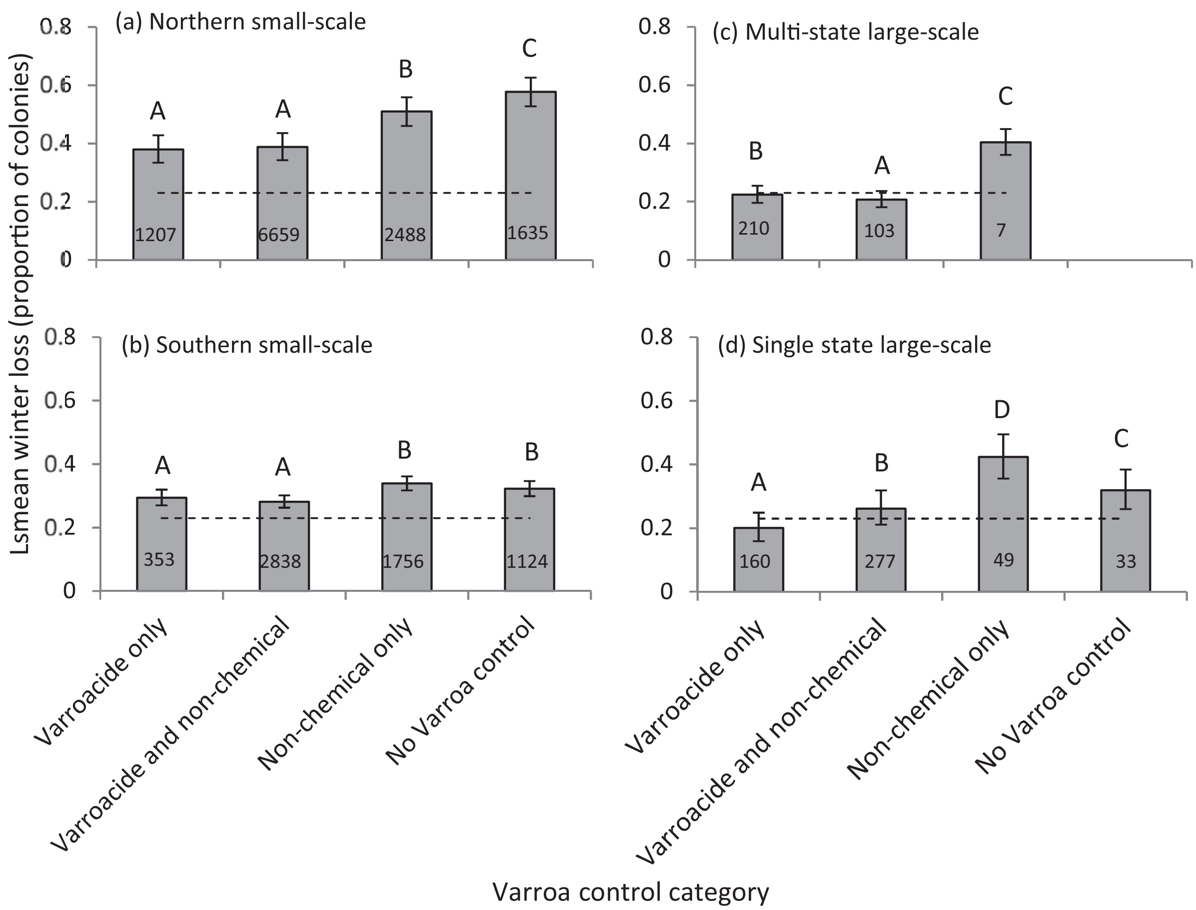

Fig. 1. Winter colony losses associated with *Varroa* control category for (a) northern small-scale, (b) southern small-scale, (c) multi-state large-scale, and (d) single-state large-scale beekeepers. The dashed line shows the grand mean winter loss. Error bars indicate 95% confidence intervals and numbers inside bars indicate sample sizes. Different letters above bars indicate losses are significantly different based on Tukey–Kramer tests.

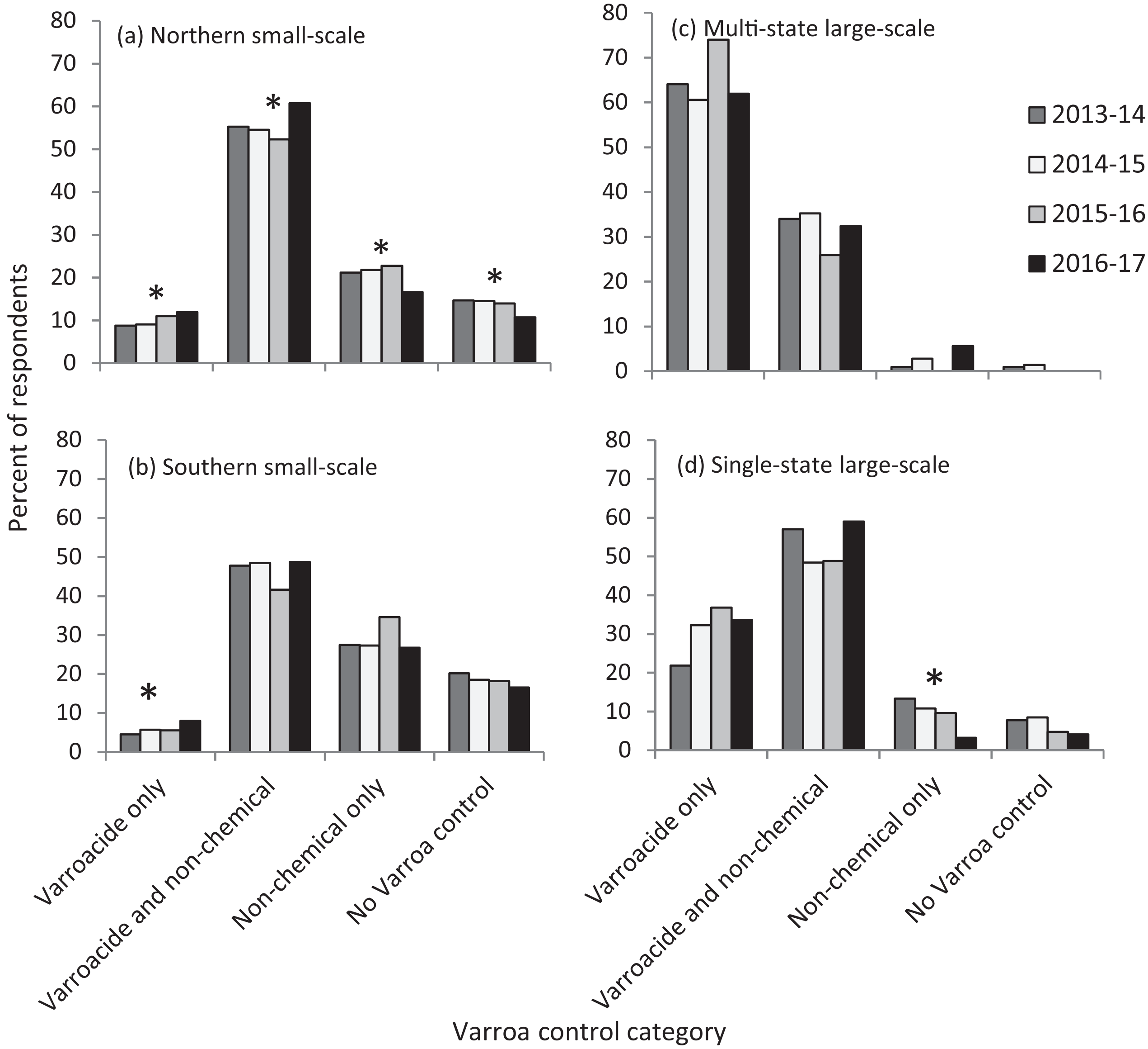

Fig. 2. Percent of (a) northern small-scale, (b) southern small-scale, (c) multi-state large-scale, and (d) single-state large-scale beekeepers each year who used varroacides only, both varroacide and nonchemical management, nonchemical management only, and neither category of *Varroa* control. Asterisks indicate a significant change (α = 0.0125) in frequency of use over survey years in logistic regression analyses.

P = 0.9580; Fig. 2c). Single-state large-scale beekeepers exhibited a significant change in use of nonchemical practices (χ^2_3 = 11.8, P = 0.0080). Specifically, reported use of nonchemical practices decreased by 75.5% from 2013–2014 to 2016–2017 (Fig. 2d).

Varroacides

Frequencies of Reported Use and Associated Winter Loss by Chemical Class

In total, 6,002; 2,226; 272; and 386 northern small-scale, southern small-scale, multi-state large-scale, and single-state large-scale beekeepers, respectively, from 2013–2014 through 2016–2017 surveys indicated they had used at least one varroacide product and provided responses regarding which products they used. Small-scale beekeepers in both regions most frequently indicated they used only organic acids. They also frequently reported using only essential oils or only synthetic chemicals. Multi-state large-scale beekeepers most frequently reported using only synthetic chemicals, followed by a combination of synthetic chemicals and organic acids. Use of each other varroacide type was reported by less than 10% of multi-state large-scale beekeepers. Exclusive use of organic acids was reported by a plurality of single-state large-scale beekeepers, followed by a combination of synthetic chemicals and organic acids, and synthetic chemicals alone.

For all operation types, winter colony losses differed among respondents who reported using different types of varroacides (northern small-scale: χ^2_6 = 246.2, P < 0.0001, southern small-scale: χ^2_6 = 93.6, P < 0.0001; multi-state large-scale: χ^2_6 = 7,820.7, P < 0.0001; single-state large-scale: χ^2_6 = 5,479.4, P < 0.0001; Fig. 3). In all operation types, the lowest losses were associated with use of synthetic chemicals. Specifically, use of a combination of synthetic chemicals and essential oil was associated with the lowest winter loss among northern small-scale and multi-state large-scale respondents (Fig. 3a and c), although this average loss for northern small-scale beekeepers was higher than the grand mean loss (Fig. 3a). Use of

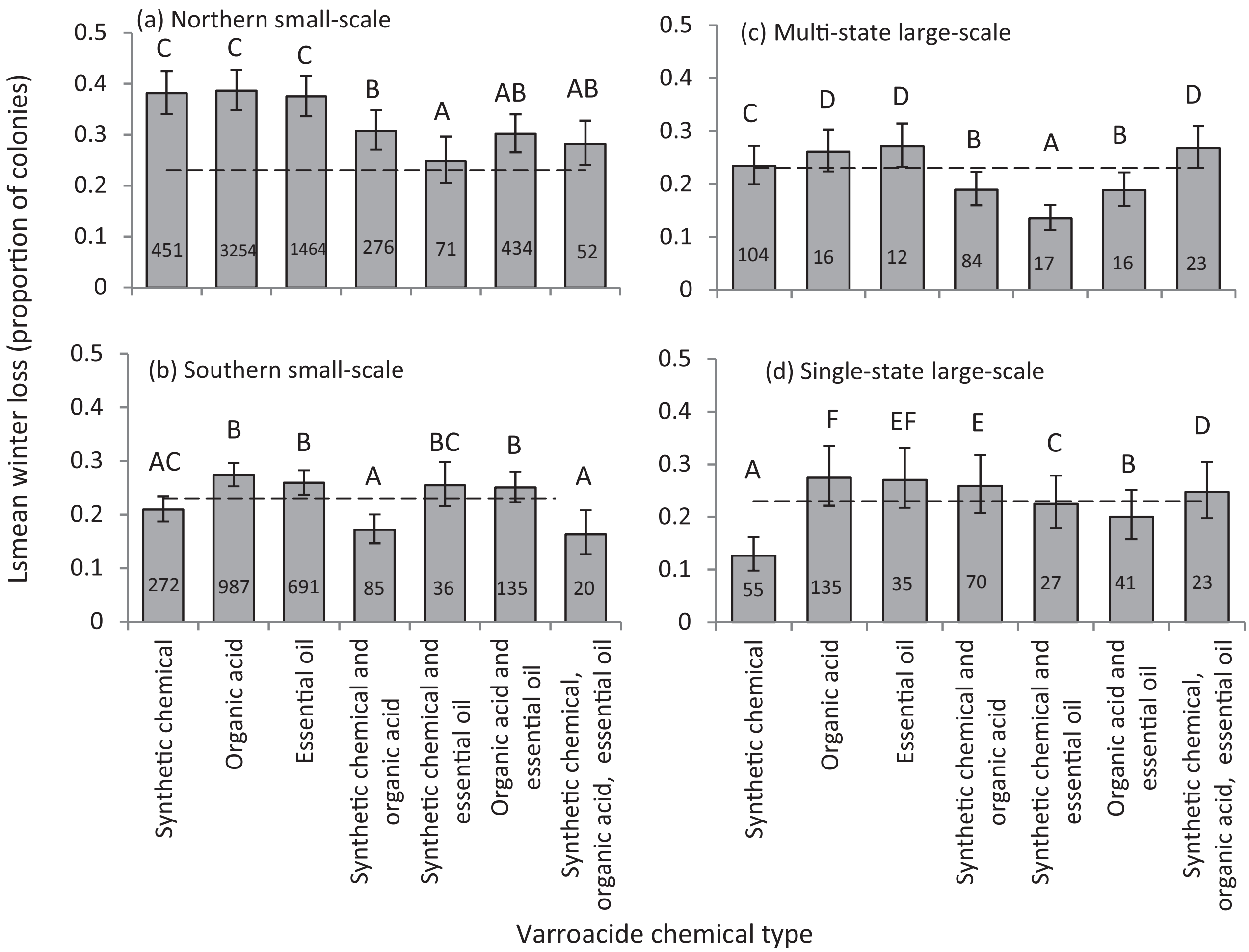

Fig. 3. Winter losses by chemical type(s) of varroacides used by (a) northern small-scale, (b) southern small-scale, (c) multi-state large-scale, and (d) single-state large-scale respondents. The dashed line shows the grand mean winter loss. Numbers inside of bars represent sample sizes. Error bars represent 95% confidence intervals. Different letters indicate lsmeans are significantly different based on Tukey–Kramer tests.

synthetic chemicals in combination with organic acids or a combination of all three varroacide types was associated with the lowest losses among southern small-scale respondents (Fig. 3b). These losses were not significantly different from the mean loss of those who reported using only synthetic chemicals, but they were significantly lower than losses for all other groups of southern small-scale respondents. For single-state large-scale beekeepers, the average loss for those who reported using synthetic chemicals alone was at least 37.0% lower than average losses of those who used other varroacide chemical types or combinations (Fig. 3d). Sole use of organic acids or essential oil was associated with highest loss rates for each group of beekeepers.

Reported Use of Varroacide Chemical Types Over Time

Both groups of small-scale beekeepers changed the types of varroacides they used over the survey time frame (northern: $\chi^2_6 = 196.8$, $P < 0.0001$; southern: $\chi^2_6 = 100.1$; $P < 0.0001$). Reported use of organic acids increased by 28.9% and 40.0% for northern and southern beekeepers, respectively (Fig. 4a and b). Reported use of essential oil in both regions decreased over time (59.8% and 50.0% decreases for northern and southern regions, respectively; Fig. 4a and b). Small-scale beekeepers in both regions also increasingly reported using a combination of synthetic chemicals and organic acids (147% and 164% increases for northern and southern regions, respectively; Fig. 4a and b), and a combination of organic acids and essential oil (76.1% and 98.2% increases for northern and southern regions, respectively; Fig. 4a and b). Neither group of large-scale beekeepers exhibited significant changes over time in reported varroacide type (multi-state: $\chi^2_6 = 4.6$, $P = 0.5905$; single-state: $\chi^2_6 = 10.6$, $P = 0.1030$; Fig. 4c and d).

Frequencies and Losses Associated With Number of Varroacide Products Reported

In total, 6,162; 2,276; 278; and 396 northern small-scale, southern small-scale, multi-state large-scale, and single-state large-scale respondents, respectively, provided information regarding the number of varroacide products they used over the course of a year. The majority of small-scale beekeepers in both regions reported using one product, and 20% or fewer respondents within each of these groups reported using any number of products greater than one. Large-scale beekeepers from both groups also most frequently reported using one varroacide product, but use of two products was nearly as frequently reported. Less than 20% of respondents in either large-scale beekeeper group reported using any number of varroacide products greater than two.

For all groups of beekeepers, average winter loss differed based on the number of varroacide products reported (northern small-scale: $\chi^2_3 = 324.4$; $P < 0.0001$; southern small-scale: $\chi^2_3 = 35.0$, $P < 0.0001$; multi-state large-scale: $\chi^2_3 = 4{,}078.7$, $P < 0.0001$;

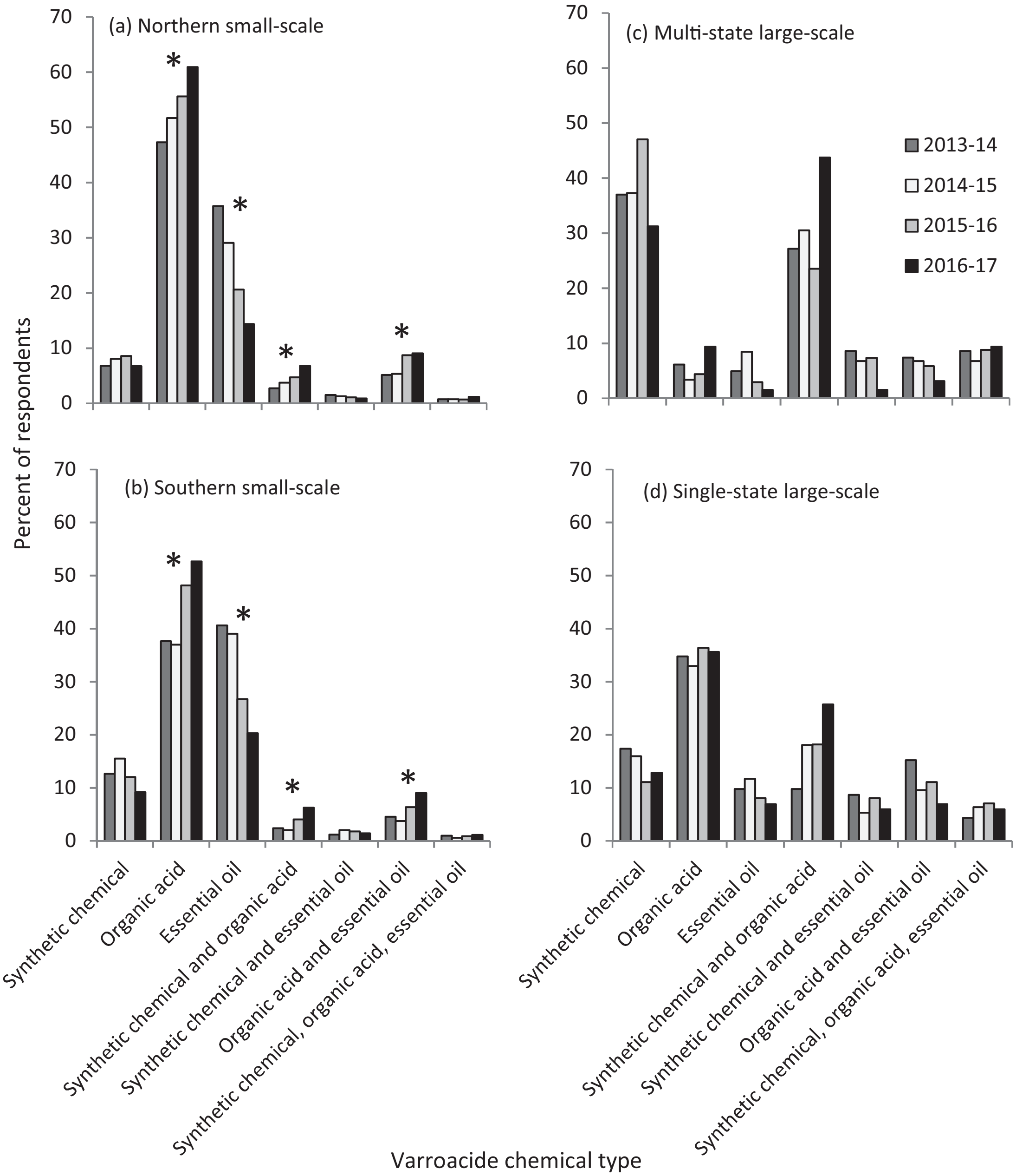

Fig. 4. Relative frequencies of (a) northern small-scale, (b) southern small-scale, (c) multi-state large-scale, and (d) single-state large-scale respondents each year who reported using varroacides from each chemical type or combinations of chemical types. Percentages are based on the number of respondents in a single year who indicated they used at least one varroacide. Asterisks indicate a significant change ($\alpha = 0.0071$) in frequency of use over survey years in logistic regression analyses.

single-state large-scale: $\chi^2_3 = 879.3$, $P < 0.0001$; Fig. 5). Average losses ranged from 39.3% to 19.4% of colonies for northern small-scale beekeepers and from 26.4% to 16.2% of colonies for southern small-scale beekeepers (Fig. 5a and b). For both of these groups, use of higher numbers of varroacide products was associated with lower average winter losses (Fig. 5a and b). Multi-state large-scale respondents who reported using two varroacide products averaged 18.7% of colonies lost, and this loss rate was at least 16.8% lower than the average losses of those who used other numbers of products (Fig. 5c). Single-state large-scale beekeepers who reported using one or four products each averaged approximately 18% of colonies lost, and these losses were at least 16.4% lower than average losses associated with use of other numbers of products (Fig. 5d).

Use and Losses Associated With a Single Varroacide Product as the Only *Varroa* Control

In total, 843 respondents (pooling all operation types) indicated they used only one varroacide product and no nonchemical practices over the course of a year. The most frequently reported products among these respondents were thymol (35.3% of respondents), formic acid (28.1% of respondents), oxalic acid (15.3% of respondents), and amitraz (8.9% of respondents).

Beekeepers who reported using an amitraz-based product averaged the lowest winter loss (18.8% loss; Fig. 6). Those who reported using oxalic acid, thymol, or formic acid averaged moderately low winter losses (32.4%, 36.8%, and 38.8% losses, respectively; Fig. 6). The least-used varroacide products (coumaphos, fluvalinate, and hop

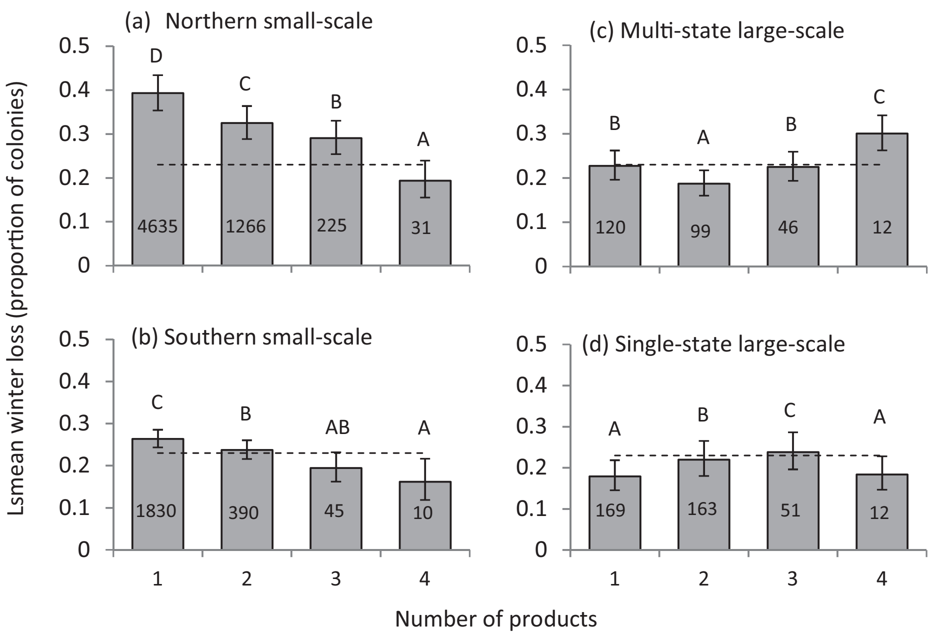

Fig. 5. Winter colony losses associated with the number of varroacide products reported over the course of 1 yr by (a) northern small-scale, (b) southern small-scale, (c) multi-state large-scale, and (d) single-state large-scale beekeepers. Percentages are calculated based on the total number of beekeepers in each operation type who used at least one varroacide product. Dashed lines indicate the grand mean winter loss. Numbers inside bars indicate sample sizes. Error bars represent 95% confidence intervals and different letters indicate lsmeans are significantly different based on Tukey–Kramer tests.

oil) were associated with the highest winter losses (Fig. 6). Among respondents who reported using only one varroacide product, average winter losses associated with all products other than amitraz were higher than the grand mean winter loss (Fig. 6).

Losses Associated With the Most Frequently Reported Combinations of Varroacides

Northern small-scale beekeepers most frequently reported using formic acid only, thymol only, oxalic acid only, a combination of formic acid and oxalic acid, and hop oil only. Approximately one-quarter of northern small-scale respondents total reported using other combinations of varroacides. Southern small-scale beekeepers most frequently reported using only single varroacide products over the course of a year (thymol, formic acid, oxalic acid, amitraz, or hop oil), with only 22.1% reporting use of combinations of products or other single products. Multi-state large-scale beekeepers most frequently reported using amitraz, followed by amitraz in combination with oxalic acid, formic acid, or thymol. Thymol alone was also frequently reported, and 37.3% of multi-state large-scale beekeepers reported using other combinations of varroacides. Single-state large-scale beekeepers most frequently reported using formic acid alone, amitraz alone, a combination of formic acid and oxalic acid, thymol alone, and a combination of amitraz and formic acid. However, nearly half of single-state large-scale beekeepers reported using other combinations of varroacides.

In all operation types, winter mortality was significantly different among groups of beekeepers who reported using different combinations of varroacides over the course of a year (northern small-scale: χ^2_5= 381.0, $P < 0.0001$; southern small-scale: $\chi^2_5 = 63.9$, $P < 0.0001$; multi-state large-scale: $\chi^2_5 = 11{,}259.0$, $P < 0.0001$; single-state large-scale: $\chi^2_5 = 3{,}142.7$, $P < 0.0001$; Fig. 7). For beekeepers in southern small-scale and single-state large-scale operations, the lowest average losses were associated with the sole use of amitraz (Fig. 7b and d). For southern small-scale beekeepers, this loss was

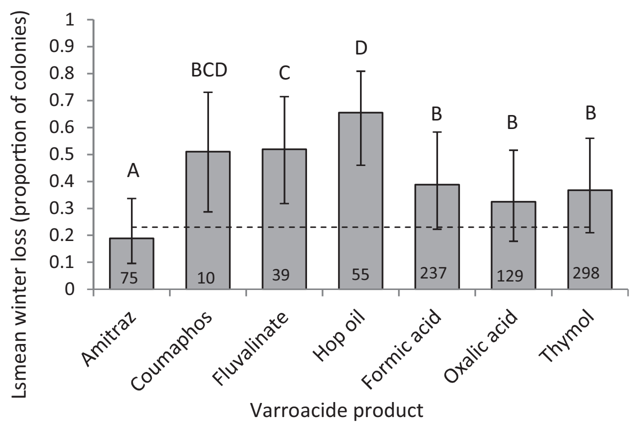

Fig. 6. Winter colony losses by varroacide product for respondents who reported using a single varroacide product as the only *Varroa* control method over the course of a year. The dashed line represents the grand mean winter loss. Numbers inside of bars indicate sample sizes. Error bars represent 95% confidence intervals. Different letters above bars indicate winter losses are significantly different based on Tukey–Kramer tests.

at least 13% lower than the average loss associated with any other varroacide or combination (Fig. 7b). For single-state large-scale beekeepers, the average loss associated with sole amitraz use was at least 33% lower than average loss associated with any other varroacide or combination. In northern small-scale and multi-state large-scale operations, the lowest average winter losses were associated with a combined use of two different products. Specifically, the group of northern small-scale beekeepers who reported using the combination of formic and oxalic acids averaged losses that were at least 11% lower than those of other groups of beekeepers in this operation type (Fig. 7a). Reported use of a combination of amitraz and thymol was associated with an average loss at least 39% lower

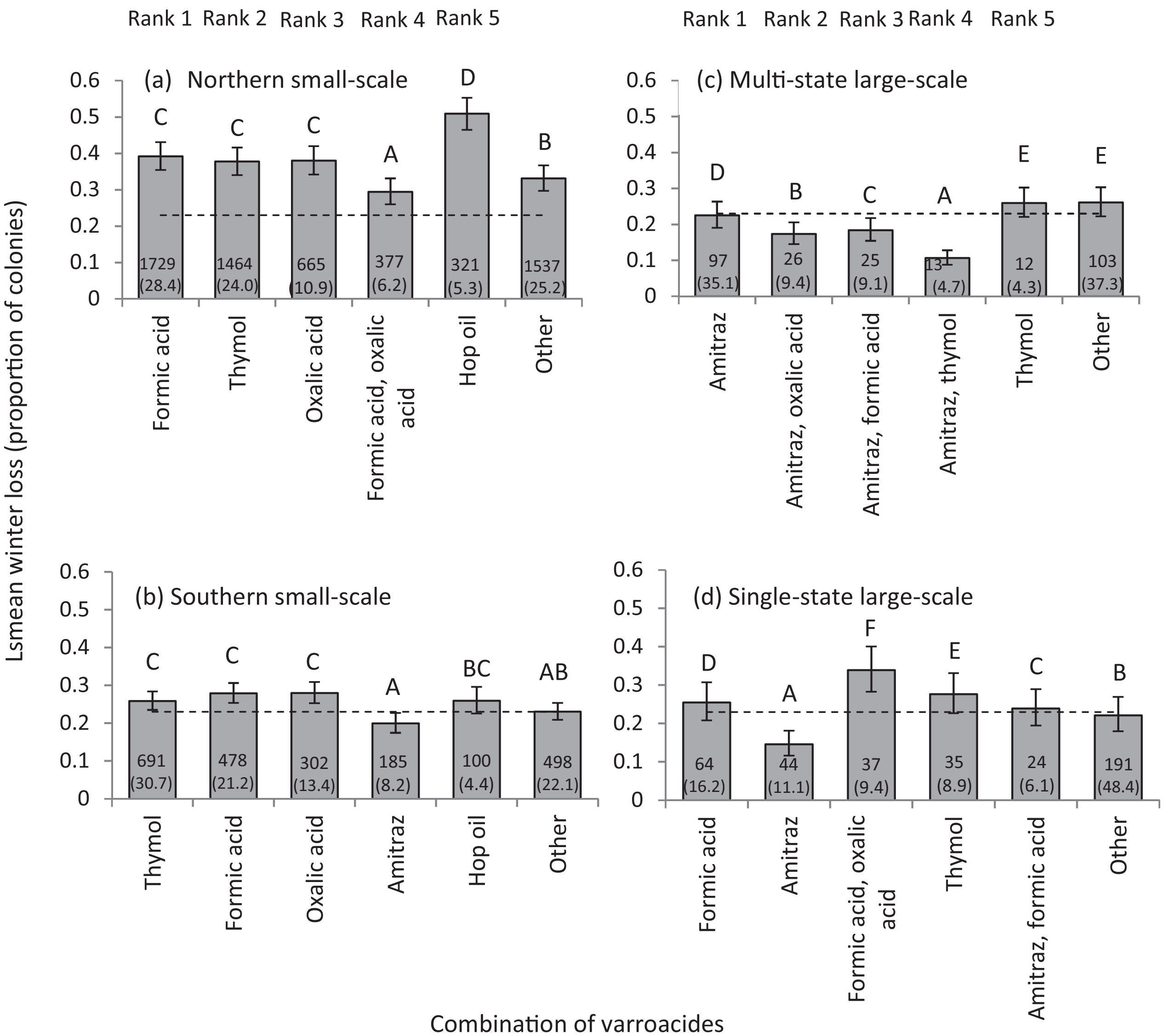

Fig. 7. Lsmean winter colony losses experienced by (a) northern small-scale, (b) southern small-scale, (c) multi-state large-scale, and (d) single-state large-scale beekeepers who indicated they used each of the five most frequently-reported combinations of varroacides within an operation type. The dashed line represents the grand mean winter loss. Numbers inside bars indicate sample sizes and numbers in parentheses indicate the corresponding percentages of respondents who reported using each combination of varroacides. Error bars represent 95% confidence intervals. Different letters indicate lsmeans are significantly different based on Tukey–Kramer tests.

than other varroacides or combinations reported by multi-state large-scale beekeepers (Fig. 7c).

Nonchemical *Varroa* Control Practices

Frequencies of Reported Use

In total, 15,495; 7,897; 394; and 631 northern small-scale, southern small-scale, multi-state large-scale, and single-state large-scale beekeepers, respectively, provided information regarding their use of six nonchemical *Varroa* control practices. Approximately half of the small-scale beekeepers in each region indicated they used screened bottom boards, and approximately one-quarter in each region reported splitting colonies (Fig. 8a and b). small-cell comb was the least frequently-reported nonchemical practice by small-scale beekeepers in each region (approximately 5% of beekeepers in each region; Fig. 8a and b). Multi-state large-scale beekeepers frequently reported that they split colonies (70.3% of respondents). Less than 20% of multi-state large-scale respondents reported using any other single nonchemical practice (Fig. 8c). Single-state large-scale beekeepers also frequently reported splitting colonies (64.0% of respondents). Use of screened bottom boards and mite-resistant stock was reported by 35.8% and 31.5%, respectively, of single-state large-scale respondents. Only 17.6% reported using drone comb removal, and few single-state large-scale respondents reported using small-cell comb or powdered sugar (Fig. 8d).

Reported Use Over T

Northern small-scale beekeepers exhibited a 25.1% increase in reported use of drone comb removal (χ^2_1 = 16.3; P < 0.0001; Fig. 9a) and a 31.8% increase in the practice of splitting colonies (χ^2_1 = 52.8; P < 0.0001; Fig. 9a) over the study period. Use of powdered sugar among northern small-scale beekeepers decreased by 22.9% (χ^2_1 = 30.7; P < 0.0001; Fig. 9a) over the same period. Among

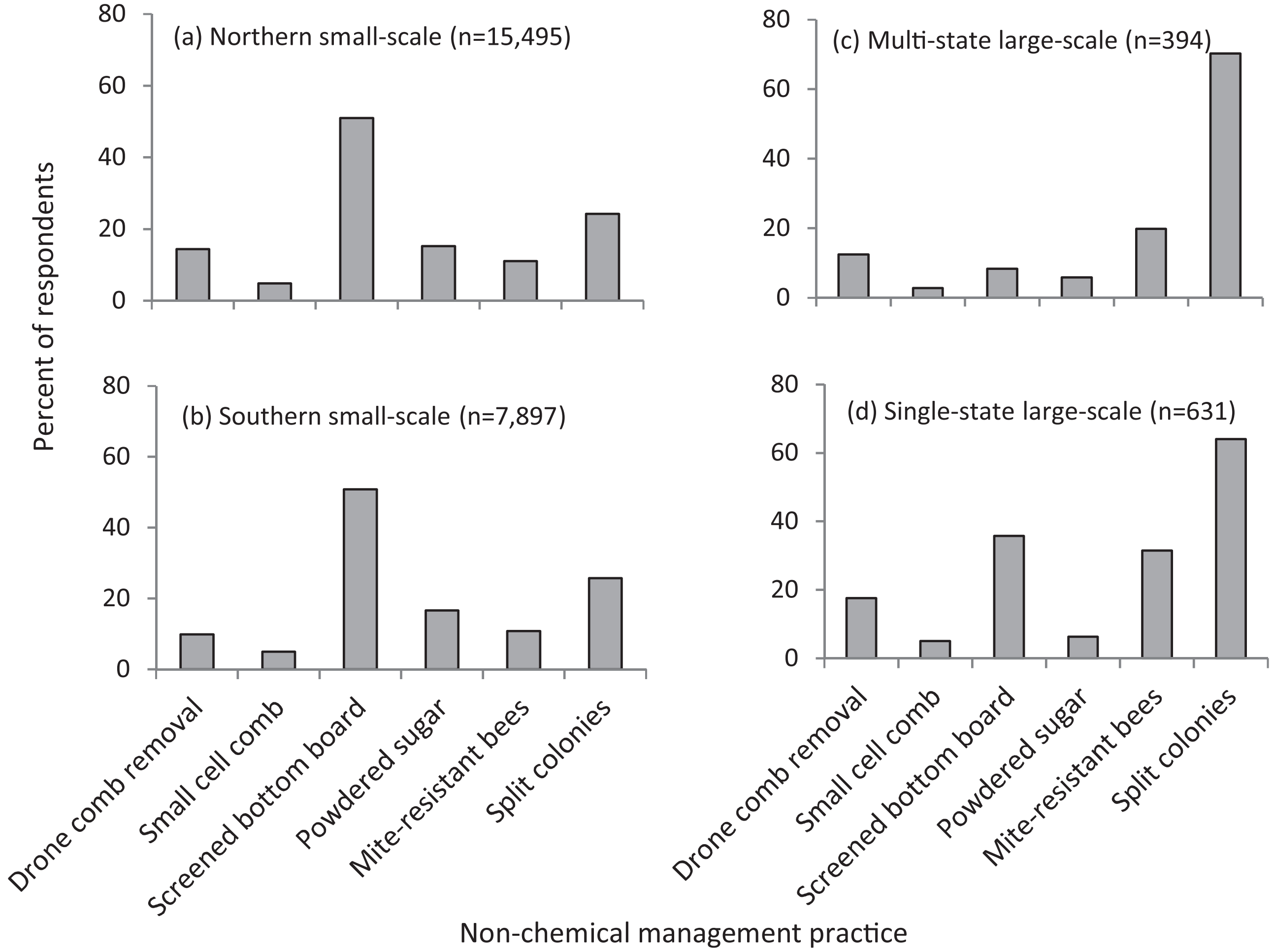

Fig. 8. Percentages of (a) northern small-scale, (b) southern small-scale, (c) multi-state large-scale, and (d) single-state large-scale respondents who reported using each nonchemical management practice in combined 2013–2014 through 2016–2017 surveys. Note that because some respondents reported using more than one practice, the percentages shown for a given operation type may sum to greater than 100.

southern small-scale beekeeper respondents, use of small-cell comb increased from 2013–2014 to 2016–2017 by 44.3% (χ^2_1 = 8.6; P = 0.0034), although less than 6% of beekeepers in this operation type reported using small-cell comb in any individual year (Fig. 9b). Reported use of powdered sugar by southern small-scale beekeepers decreased over time by 27.2% (χ^2_1 = 21.2; P < 0.0001; Fig. 9b), and reported use of mite-resistant stock decreased by 18.6% (χ^2_1 = 8.6; P = 0.0034). Among multi-state large-scale beekeeper respondents, reported use of drone comb removal increased over time by 182% (χ^2_1 = 7.7; P = 0.0055; Fig. 9c). Single-state large-scale beekeepers exhibited no significant changes over time in use of any nonchemical practice (Fig. 9d).

Frequencies and Losses Associated With the Number of Nonchemical Practices Reported

In total, 9,802; 4,955; 299; and 483 northern small-scale, southern small-scale, multi-state large-scale, and single-state large-scale beekeepers, respectively, specified which specific nonchemical management practices they used. Small-scale beekeepers in each region exhibited similar patterns in number of practices reported, with approximately 42% of respondents from each region reporting use of one practice and fewer respondents reporting use of higher numbers of practices. Multi-state large-scale beekeepers also most frequently reported using fewer practices, with an even higher percentage (63.5%) relative to small-scale beekeepers reporting use of only one practice. Single-state large-scale beekeepers most frequently reported using one or two nonchemical practices over the course of a year (33.7% and 34.8% reported one and two practices, respectively).

Respondents from all operation types exhibited differences in winter loss associated with the number of nonchemical practices they reportedly used (northern small-scale: χ^2_5 = 266.8, P < 0.0001; southern small-scale: χ^2_5 = 64.0, P < 0.0001; multi-state large-scale: χ^2_3 = 9,140.6, P < 0.0001; single-state large-scale: χ^2_4 = 3,523.3, P < 0.0001). Northern small-scale beekeepers who reported using three, four, or six nonchemical practices over the course of a year averaged the lowest losses within this operation type (30–39% of colonies lost; Fig. 10a), and southern small-scale beekeepers who reported using two to five nonchemical practices averaged the lowest losses within this operation type (27–31% of colonies lost; Fig. 10b). Winter losses were significantly different among all numbers of products used by multi-state large-scale beekeepers (Fig. 10c), with those who reported using three practices averaging the lowest loss (14.6%). Single-state large-scale beekeepers who reported using one nonchemical practice averaged 18.5% of colonies lost, and this was significantly lower than the mean losses of those who reported any other number of nonchemical practices (Fig. 10d).

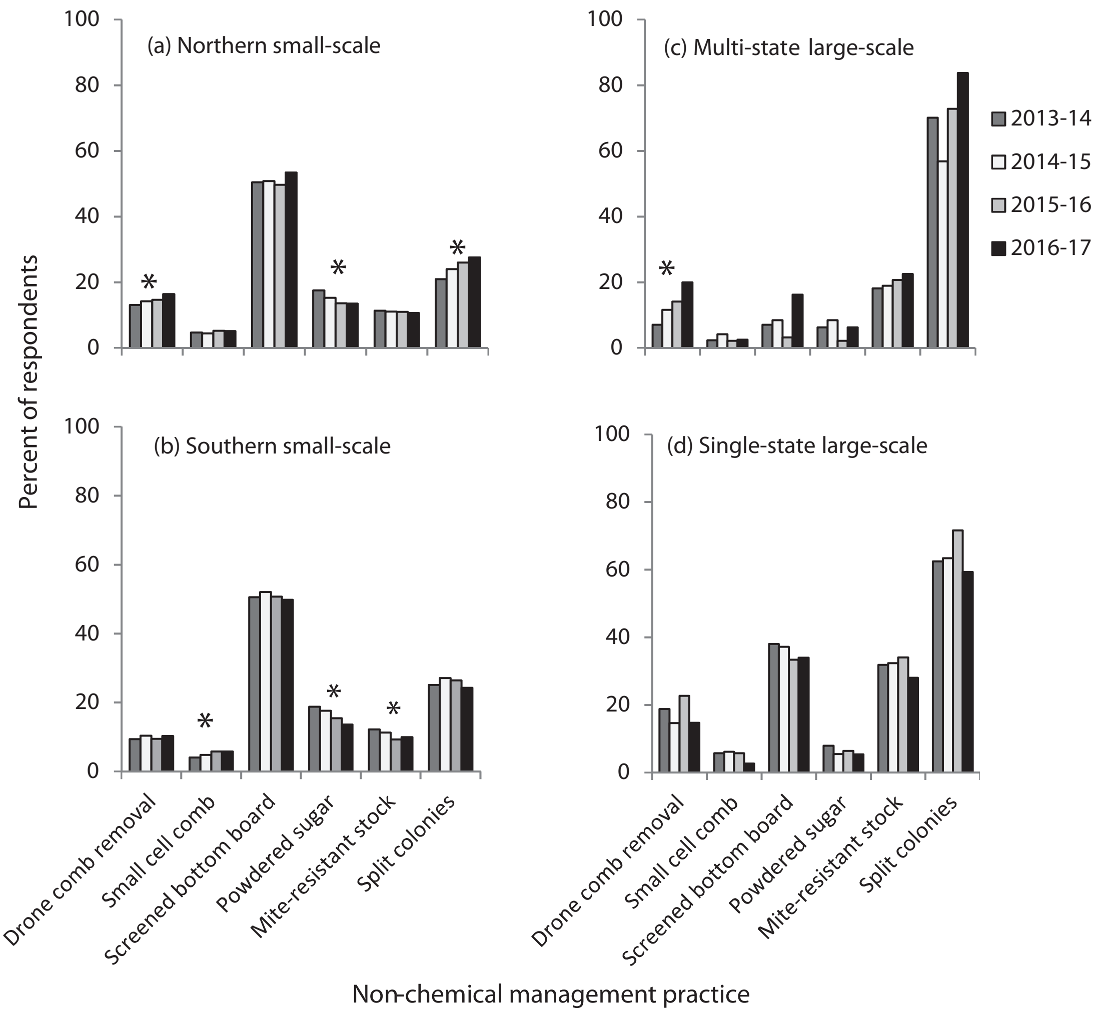

Fig. 9. Percent of (a) northern small-scale, (b) southern small-scale, (c) multi-state large-scale, and (d) single-state large-scale beekeepers each year who indicated they had used each of six nonchemical management practices to control *V. destructor* populations. Asterisks indicate a significant trend in use over time based on a Cochran–Armitage test.

Use and Losses Associated With a Single Nonchemical Practice as the Only *Varroa* Control

Among the 3,459 respondents who indicated they used a single nonchemical management practice and no varroacides, 59.8% indicated they used screened bottom boards. Reported use of any other nonchemical practice as the sole *Varroa* control method was much less frequent, with the second most common practice (splitting colonies) reported by only 15% of respondents.

Average winter losses for respondents who reported using only a single nonchemical practice to control *Varroa* were higher than the grand mean winter loss, regardless of the nonchemical practice they reported using. Those who reported splitting colonies averaged the lowest winter loss (32.8% loss), and this loss was significantly different from losses of those who used all other nonchemical practices other than small-cell comb (Fig. 11). Those who reported using drone comb removal or powdered sugar averaged approximately 62% of colonies lost, and these losses were significantly higher than losses of those who reported using all other nonchemical practices (Fig. 11).

Frequencies and Losses Associated With the Reported Use of Mite-Resistant Bee Stock

In total, 11,378; 5,801; 302; and 491 northern small-scale, southern small-scale, multi-state large-scale, and single-state large-scale beekeepers, respectively, provided information regarding the breeds or lines of *A. mellifera* they used in their operations. In all operation types, use of nonresistant lines was reported more frequently than use of mite-resistant lines, and use of a combination of mite-resistant and nonresistant lines within the same operation was reported more frequently than use of only mite-resistant lines. Among respondents in any operation type who reported using exclusively mite-resistant or exclusively nonresistant lines, use of a single line of *A. mellifera* was reported more frequently than use of multiple lines. Small-scale beekeepers in both regions, as well as multi-state large-scale beekeepers, most frequently reported using a single line of nonresistant bees, whereas single-state large-scale beekeepers reported using a combination of mite-resistant and nonresistant lines as frequently as they reported using a single nonresistant line.

All operation types exhibited significantly different winter losses associated with the mite-resistant status of genetic lines reported (northern small-scale: χ^2_4 = 237.4, P < 0.0001; southern small-scale: χ^2_4 = 24.4, P < 0.0001; multi-state large-scale: χ^2_3 = 5,801.7, P < 0.0001; single-state large-scale: χ^2_3 = 4,453.1, P < 0.0001). In both regions of small-scale operations, use of multiple mite-resistant lines in the same operation was associated with the lowest average loss. This was significantly lower than the mean losses for groups of beekeepers who reported using only nonresistant stock, but not

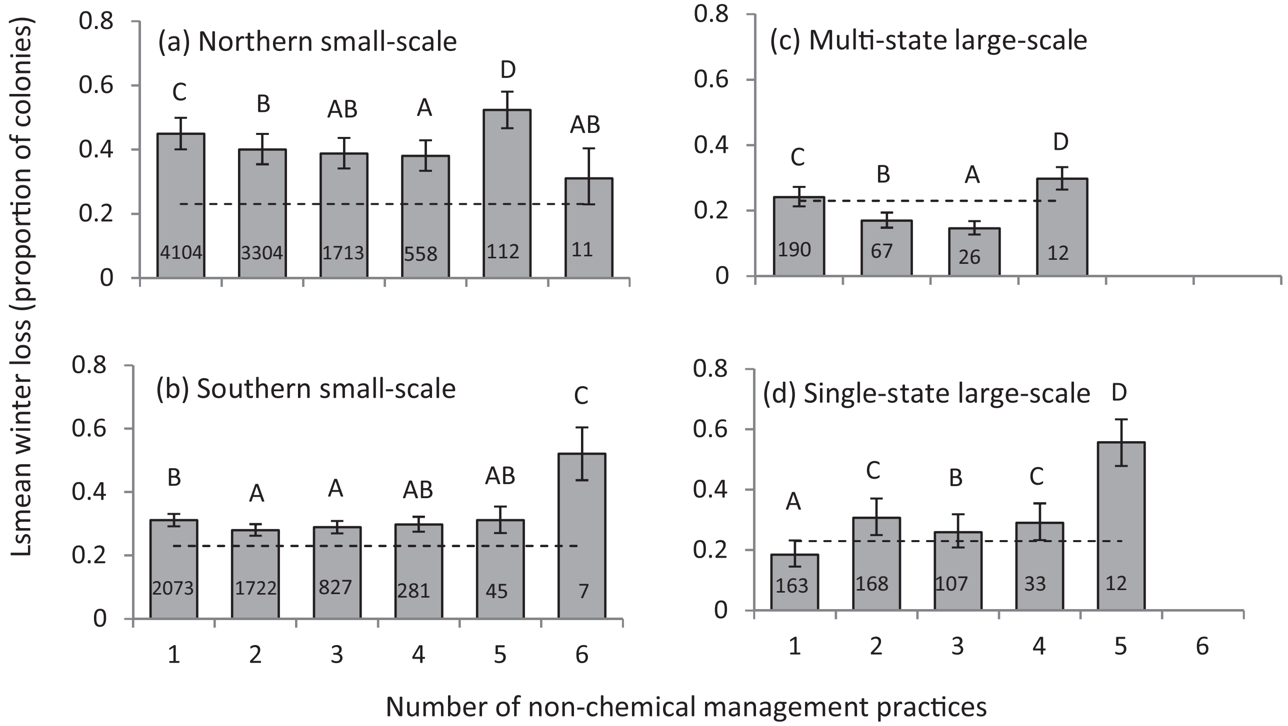

Fig. 10. Winter colony losses by number of nonchemical *Varroa* control practices reported by (a) northern small-scale, (b) southern small-scale, (c) multi-state large-scale, and (d) single-state large-scale respondents. The dashed line represents the grand mean winter loss. Numbers inside bars indicate sample sizes. Error bars represent 95% confidence intervals and different letters indicate lsmeans are significantly different based on Tukey–Kramer tests.

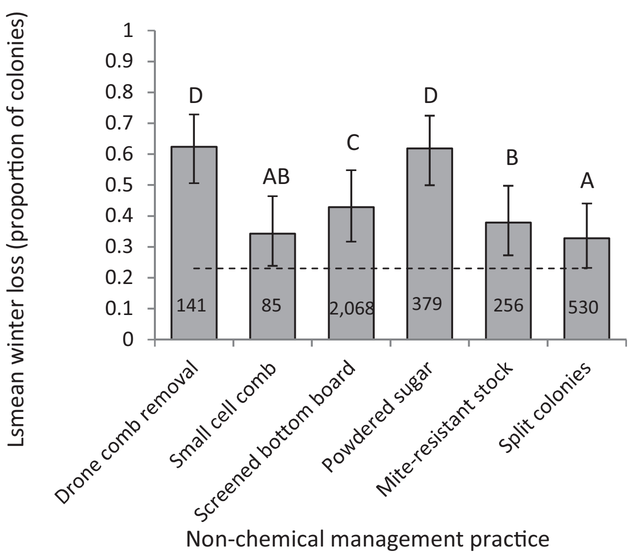

Fig. 11. Winter colony losses by nonchemical management practice for respondents who reported using a single nonchemical practice and no varroacides over the course of a year. The dashed line represents the grand mean winter loss. Numbers inside of bars indicate sample sizes. Error bars represent 95% confidence intervals. Different letters above bars indicate winter losses are significantly different based on Tukey–Kramer tests.

significantly different from those who reported using a single mite-resistant line or a combination of mite-resistant and nonresistant lines (Fig. 12a and b). For multi-state large-scale beekeepers, all pairwise differences in average winter loss were significant (Fig. 12c). Within this operation type, those who reported using a combination of mite-resistant and nonresistant lines exhibited the lowest mean loss, whereas those who used only nonresistant lines exhibited the highest mean losses (Fig. 12c). All groups of single-state large-scale beekeepers also exhibited significantly different winter losses from one another. However, the lowest losses within this operation type were associated with use of nonresistant stock and the highest losses with use of mite-resistant stock (Fig. 12d).

Losses Associated With Frequently Reported Combinations of Nonchemical Practices

The most frequently reported combinations of nonchemical practices used by small-scale beekeepers almost always included screened bottom boards, whereas the most frequently reported combinations reported by large-scale beekeepers almost always included splitting colonies (Fig. 13). Average winter colony losses among groups of respondents who reported using different combinations of nonchemical practices were significantly different for all operation types (northern small-scale: $\chi^2_5 = 359.9$, $P < 0.0001$; southern small-scale: $\chi^2_5 = 168.1$, $P < 0.0001$, multi-state large-scale: $\chi^2_5 = 9{,}353.7$, $P < 0.0001$; single-state large-scale: $\chi^2_5 = 3{,}714.9$, $P < 0.0001$). Among both groups of small-scale beekeepers and multi-state large-scale beekeepers, powdered sugar use was associated with the highest losses. Small-scale beekeepers who reported powdered sugar combined with screened bottom boards averaged the highest losses within each region, and multi-state large-scale beekeepers who reported powdered sugar combined with splitting colonies averaged the highest loss within this operation type (Fig. 13). Splitting colonies was associated with the lowest losses in all operation types (Fig. 13). The northern large-scale beekeepers who reported splitting colonies combined with using screened bottom boards averaged significantly lower losses than other groups within this operation type (Fig. 13a).

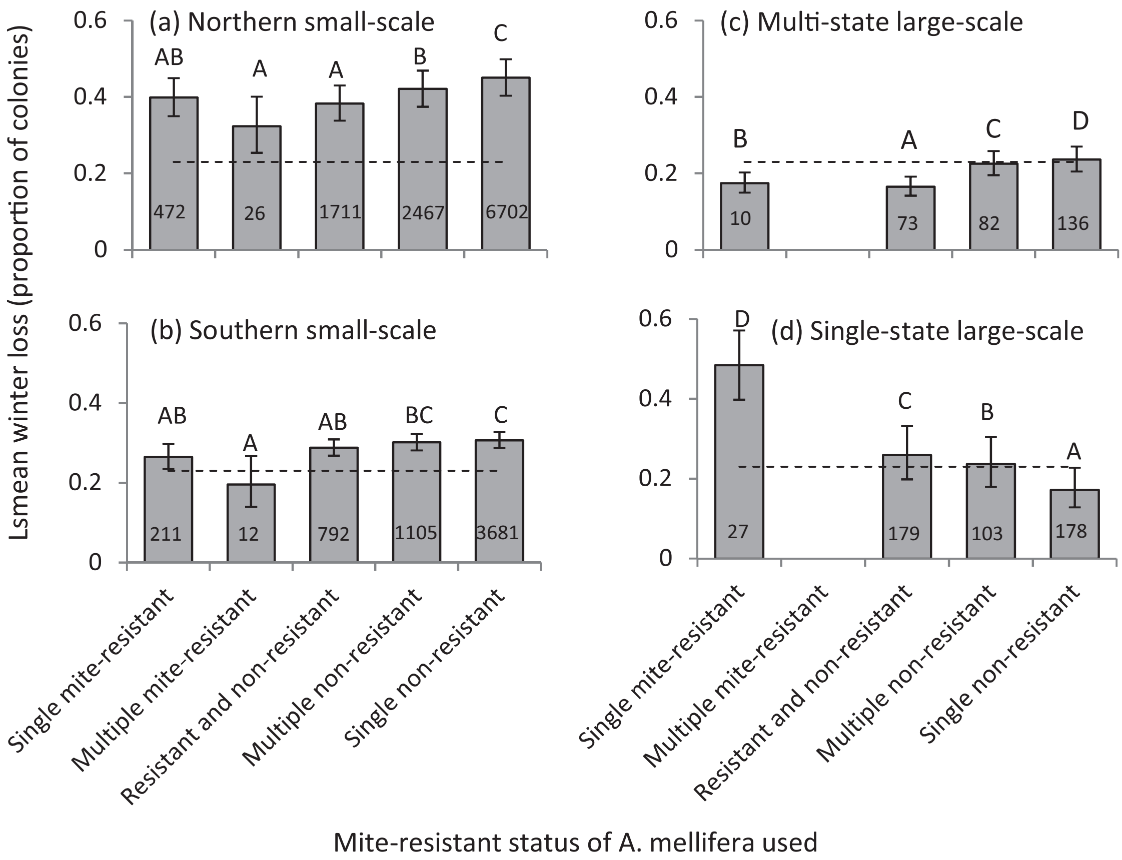

Fig. 12. Winter losses by mite-resistant status of *A. mellifera* reported by (a) northern small-scale, (b) southern small-scale, (c) multi-state large-scale, and (d) single-state large-scale respondents. Percentages are based on the total number of respondents in each operation type who provided information regarding the race(s) of bees used. The dashed line indicates the grand mean winter loss. Error bars indicate 95% confidence intervals and numbers inside of bars indicate sample sizes. Different letters above bars indicate lsmeans are significantly different according to Tukey–Kramer tests.

Among southern small-scale beekeepers, splitting colonies combined with using screened bottom boards and splitting colonies alone were associated with significantly lower losses than any other reported combination of nonchemical practices (Fig. 13b). Among multi-state large-scale beekeepers, the lowest mean winter loss was associated with splitting colonies combined with use of mite-resistant stock (Fig. 13c). The single-state large-scale beekeepers who reported only splitting colonies averaged significantly lower winter losses than those who reported any other combination of nonchemical practices. Moreover, the highest mean loss within this operation type was exhibited by those who reported using only screened bottom boards, and this was the only frequently reported practice or combination of practices within this operation type that did not include splitting colonies (Fig. 13d).

Discussion

Our results from a survey of beekeepers in the United States demonstrated that small-scale beekeepers were less likely than large-scale beekeepers to use any *Varroa* control methods. Among beekeepers who did use some method of *Varroa* control, small-scale beekeepers commonly reported using nonchemical methods, whereas large-scale beekeepers more frequently reported using varroacides. Regardless of operation type, groups of beekeepers who reported using varroacides averaged lower winter colony mortality than groups who did not use varroacides, with use of amitraz being associated with lower losses than other varroacide products. We found that splitting colonies was associated with lower losses than other nonchemical practices, although our results suggest that nonchemical practices have limited success as stand-alone controls. Our results provide insight into the benefits and limitations of different *Varroa* control methods and support other studies that have suggested it is best to integrate different *Varroa* control methods into successful management plans (Boecking and Genersch 2008, Giacobino et al. 2016).

Note that we did not obtain information on *Varroa* loads from survey respondents, and so we do not know to what extent colony losses were explained by *Varroa*. We also cannot draw a direct link between any *Varroa* control practices and winter colony losses, as our data are observational. In addition, our data may not be representative of the U.S. beekeeping population because they are not from a random sample of beekeepers, and they may be biased or inaccurate because they are self-reported. Despite these limitations, our results provide insight into which *Varroa* control methods are most commonly used and which methods may be most effective. This will be able to inform future studies that aim to improve *Varroa* management practices.

The majority of large-scale beekeepers in our study indicated they used at least one varroacide, whereas many small-scale beekeepers reported using exclusively nonchemical control practices or did not use any *Varroa* control. Winter mortality was lower overall for large-scale beekeepers than for small-scale beekeepers, as reported previously (Lee et al. 2015, Seitz et al. 2016, Kulhanek et al. 2017,

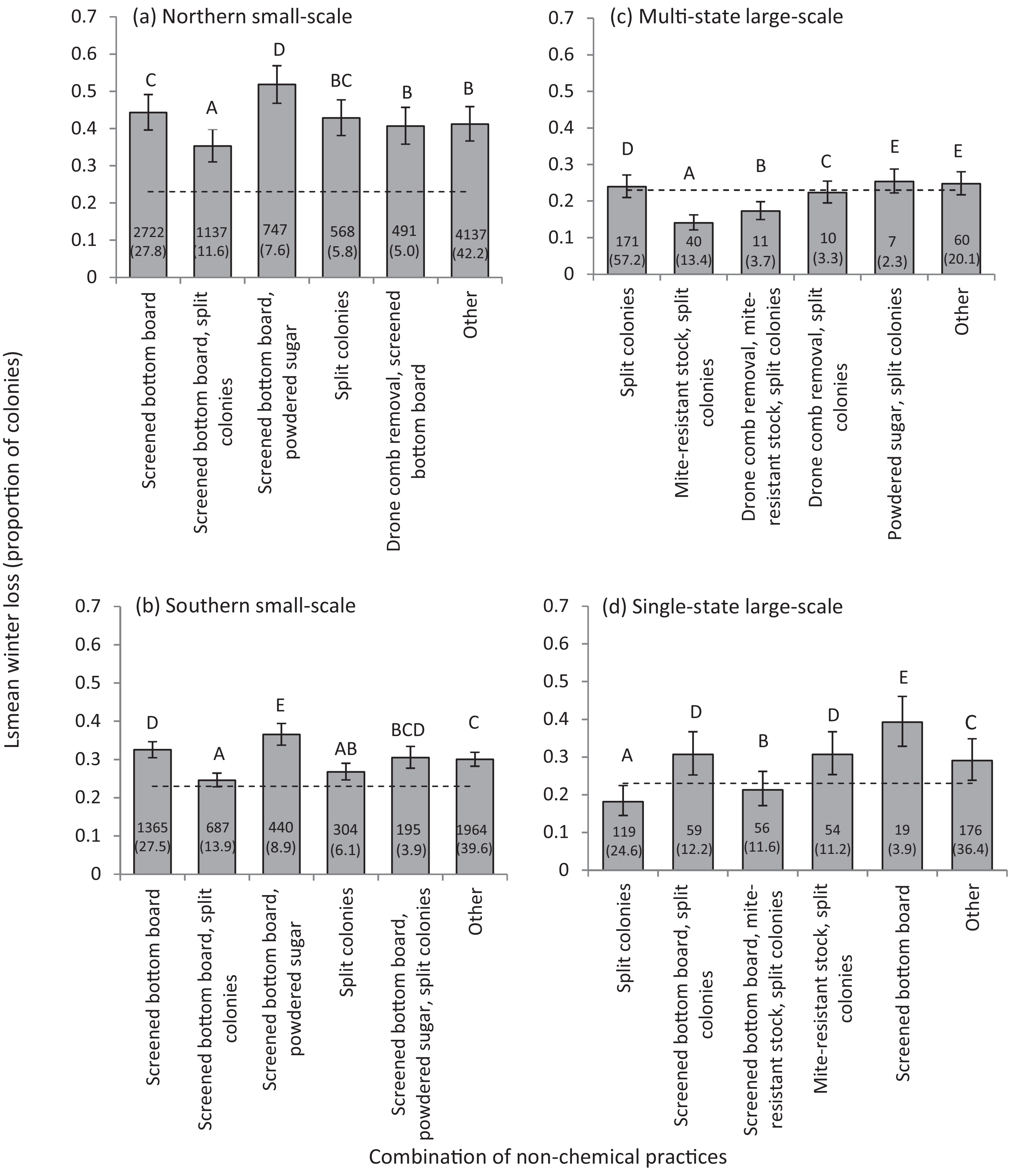

Fig. 13. Lsmean winter colony losses for (a) northern small-scale, (b) southern small-scale, (c) multi-state large-scale, and (d) single-state large-scale respondents who indicated they used each of the five most frequently-reported combinations of nonchemical management practices within an operation type. The dashed line represents the grand mean winter loss. Numbers inside bars indicate sample sizes and numbers in parentheses indicate the corresponding percentages of respondents who reported using each combination of practices. Error bars represent 95% confidence intervals. Different letters indicate lsmeans are significantly different based on Tukey–Kramer tests.

Steinhauer 2017). Regardless of operation type, average winter mortality was lowest among beekeepers who reported using varroacides, suggesting that varroacide use is necessary for maintaining viable colonies. We also found that over the 4-yr span of the survey, increasing percentages of both groups of small-scale beekeepers reported using varroacides only. This could represent an increase in willingness of small-scale beekeepers to use varroacides, perhaps resulting from greater awareness of the impacts of *Varroa* on honey bee colonies (Kulhanek et al. 2017).

Among the respondents who reported using varroacides, multi-state large-scale beekeepers most frequently reported using synthetic chemicals, whereas respondents from all other operation types most frequently reported using organic acids. Furthermore, the reported use of organic acids by small-scale beekeepers in both regions increased over time when used as the only chemical class and when used in addition to a synthetic chemical or essential oil. This increase is likely driven by increased use of oxalic acid, as it was first registered for use against *Varroa* by the U.S. Environmental Protection Agency in March 2015 (Environmental Protection Agency 2015).

Our analyses of individual varroacides used as the sole *Varroa* control method and our analyses of the most frequently used combinations of varroacides indicated that amitraz was consistently associated with the lowest winter colony losses. Amitraz has been found to be effective in both laboratory and field experiments (Gregorc et al. 2018). It therefore seems possible that use of amitraz played a role in the lower winter losses experienced by many of the respondents in our study. However, resistance to amitraz has been documented in *Varroa* populations in the United States (Elzen et al. 2000, Sammataro et al. 2005) and elsewhere (Maggi et al. 2010, Kamler et al. 2016). Moreover, we found that among multi-state large-scale beekeepers, amitraz as a stand-alone treatment was associated with higher mortality than amitraz paired with another varroacide product. Thus, despite the evidence that use of amitraz is associated with lower winter colony mortality, it is important that beekeepers not rely solely on amitraz to control *V. destructor* populations in colonies.

The three least-reported solely-used varroacides—coumaphos, fluvalinate, and hop oil—were associated with the highest winter losses among individual varroacide products. Resistance to coumaphos and fluvalinate has been documented in the United States (Pettis 2004, Gonzalez-Cabrera et al. 2016) and these products are no longer thought to be effective (Oldroyd 2007). Hop oil has been found to be insufficient for long-term *Varroa* control, particularly when brood is present (Vandervalk et al. 2014). The low reported use of these products suggests that beekeepers are aware of their low efficacy and tend to use alternative methods for *Varroa* control.

We found that use of more than one varroacide chemical type was generally associated with lower losses than use of a single chemical type. Likewise, our results with regard to the number of varroacide products reported suggest that for small-scale beekeepers, use of higher numbers of products was associated with lower winter mortality, although sample sizes for higher numbers of products were relatively low. The lower losses associated with use of more products could be associated with use of a greater diversity of chemical classes, but it could also be confounded by the number of treatment applications (Rosenkranz et al. 2010). Moreover, our analyses of chemical types and number of products did not take into account other factors that could influence winter losses such as other management practices that were used (Boecking and Genersch 2008, Giacobino et al. 2016), or environmental factors (Döke et al. 2015, Asensio et al. 2016). Indeed, northern small-scale beekeepers in our study averaged higher winter mortality than beekeepers in other operation types even when they used the same varroacide products or combinations of products, indicating that colony survival is dependent on more than use or lack of use of a product or products. The timing of treatment application can play a role in treatment efficacy (Beyer et al. 2018), and investigating associations between treatment timing and winter colony losses is a goal of future work.

Although varroacides are known to aid in the control of *Varroa* populations, we cannot rule out the possibility that sublethal doses of these chemicals negatively impact honey bees as well. Amitraz, coumaphos, and fluvalinate have all been found to accumulate in colony wax (Mullin et al. 2010), and exposure to sublethal doses of these varroacides has been found to increase honey bee mortality (De Mattos et al. 2017). Varroacides can also compromise natural defenses of *A. mellifera* against *V. destructor* by reducing and delaying grooming behavior in response to the presence of a mite (De Mattos et al. 2017). Moreover, amitraz is known to increase glutathione S-transferase activity in honey bee larvae, pupae, and nurse bees, indicating that exposure to amitraz induces toxic stress (Loucif-Ayad et al. 2008). Sublethal doses of coumaphos have been demonstrated to reduce the amount of trophallaxis by honey bees, which can compromise distribution of food within a colony (Bevk et al. 2012). Fluvalinate was found to negatively impact olfactory learning and memory in honey bees (Frost et al. 2013), impairing the ability of bees to detect floral odors, and therefore, nectar and pollen sources. The sublethal effects of varroacides on honey bees, in addition to the potential for *V. destructor* to develop resistance to varroacides (Milani 1999, Pettis 2004, Sammataro et al. 2005, Johnson et al. 2010), certainly motivate the need for a more holistic approach to controlling *V. destructor* populations.

Examining winter losses associated with individual nonchemical practices used as the only *Varroa* control method, we found that regardless of which nonchemical practice was used, average winter colony losses were higher than the grand mean winter loss. This suggests that nonchemical practices may be insufficient on their own for *Varroa* control, as other studies have demonstrated. For example, we found that exclusive use of drone brood removal was associated with the highest mean loss among all nonchemical practices, supporting previous findings that implementation of drone brood removal in the spring required a follow-up chemical treatment in the fall (Wantuch and Tarpy 2009). We found that small-cell comb was associated with low winter losses as compared with other nonchemical practices. This was unexpected, as empirical work has demonstrated that small-cell comb is ineffective at controlling *Varroa* populations (Ellis et al. 2009a, Berry et al. 2010). However, we note that, in addition to the mean loss for small-cell comb users being higher than the grand mean, the sample size for this group was relatively small, and so we do not suggest that our results refute earlier findings. Dusting with powdered sugar also previously failed to reduce *Varroa* populations (Ellis et al. 2009b, Berry et al. 2012), and here we found that the group that used powdered sugar averaged among the highest mortality rates. However, reported use of powdered sugar decreased over time in both groups of small-scale beekeepers, which may result from increasing awareness among these groups that powdered sugar is not an effective control method.

We found that in three of the four operation types, use of mite-resistant stock was associated with lower winter losses than use of nonresistant stock. In previous work, colonies bred for hygienic behavior exhibited reduced mite population growth relative to colonies not bred for hygienic behavior when mite levels were relatively low (Spivak and Reuter 2001), and colonies of mite-resistant lines required fewer miticide treatments to control *Varroa* populations than those with nonresistant lines (Ward et al. 2008). This finding therefore supports previous work suggesting that use of resistant *A. mellifera* lines is beneficial if used as part of an integrated pest management program to control *Varroa* (Delaplane et al. 2005, Tarpy et al. 2007). Our finding that use of multiple resistant lines in the same operation was associated with the lowest loss is of note. To our knowledge, no studies have investigated the effects of increased genetic diversity (i.e., use of more than one genetic line) of *A. mellifera* within a beekeeping operation. In light of our findings, this is worthy of future investigation.

We found that splitting colonies was associated with the lowest winter mortality when compared to other nonchemical *Varroa* management tools. These lower losses may result from interruptions in colonies' brood cycles, which impede *Varroa* reproduction (Evans 2015, Milbrath 2017, Cornell University College of Agriculture and Life Sciences 2018). However, splitting colonies, by definition, increases the number of colonies in an apiary. Thus, it is also possible that by splitting colonies, beekeepers replaced some or all of their lost colonies, which would have reduced our calculated loss rate for their operations. Thus, the true benefit of splitting colonies, with respect to its effect on *Varroa* control, may be inflated.

Given that *Varroa* infestations are correlated with honey bee colony mortality, it is not surprising that we found that differences in *Varroa* control methods were associated with different colony loss rates. However, many factors, including the external temperature, brood conditions, and frequency or duration of application, can influence the efficacy of varroacides and nonchemical controls (Currie and Gatien 2006, Milbrath 2017). For optimal control, beekeepers must consider environmental limitations when choosing mite control methods. Unexplored in this work are the effects of treatment application timing and dosing, and investigating these factors is a goal of our future study. Nevertheless, our findings reinforce those of other studies suggesting that varroacides may be a necessary component of management in many beekeeping operations. These results should inform experiments that directly test the efficacy and possible risks of different *Varroa* control practices so that they can be incorporated into a diversified management plan that optimizes long-term colony health and survival.

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